Cyclodextrins-Enabling Excipients: Their Present and Future Use in Pharmaceuticals

with compliments,

Diane O. Thompson
Critical Reviews in Therapeutic Drug Carrier Systems, 14 (1):1-104 (1997)

Diane O. Thompson

Critical Reviews™ in Therapeutic Drug Carrier Systems, 14(1):1–104 (1997)

Cyclodextrins—Enabling Excipients: Their Present and Future Use in Pharmaceuticals

Diane O. Thompson
CyDex, L. C., 7304 West 130th Street, Suite 370, Overland Park, KS 66213-2627

Referee: Allan R. Hedges, Ph.D., Cerestar, Hammond, IN 46320

ABSTRACT: Cyclodextrins (CDs) complex hydrophobic drugs, increasing their aqueous solubility and stability. CD complexation enables the creation of formulations for water-insoluble drugs that are difficult to deliver with more traditional formulations. Currently, 10 pharmaceutical products are marketed as CD formulations. A CD-based formulation, like any other, is evaluated for quality and safety. The 6 CDs currently available for use in pharmaceutical products are α-, ß-, and γ-CD and the methyl (M), hydroxypropyl (HP), and sulfobutylether (SBE) derivatives of ß-CD. The structural features of these CDs are evaluated for their affect on complexation performance. Optimal specifications, quality production, and safety of each CD is presented. The current and future regulatory process facing excipients is summarized, and the current regulatory status of the CDs in Japan, the United States, and Europe is presented.

KEYWORDS: CD, drug delivery, excipient, solubilization, stabilization.

TABLE OF CONTENTS

0743-4863/97 $5.00

I. INTRODUCTION

A. Cyclodextrins in Pharmaceutical Products

A drug molecule needs to be water soluble to be readily delivered to the cellular membrane, but it needs to be hydrophobic to cross the membrane. Of the two properties, water solubility is the more elusive in the complex organic structures typically found in pharmaceutical agents. Traditional formulation systems for insoluble drugs involve a combination of organic solvents, surfactants, and extreme pH conditions. These formulations are often irritating to the patient and may cause adverse reactions. At times, these methods are inadequate for solubilizing enough drug for a parenteral formulation.

Another formulation method for increasing water solubility involves the use of cyclodextrins (CDs). CDs are cyclic carbohydrates known to form complexes with hydrophobic drugs, improving their aqueous solubility. This property enables the creation of formulations for water-insoluble drugs typically difficult to formulate and deliver with more traditional additives.

The global research community has explored the use of CDs to solve numerous formulation problems for over 30 years. Biennial international conferences[1-7] and reviews[8-11] have presented the latest research in producing, characterizing, and utilizing CDs in biomedical products, foods, and cosmetics. A search of the literature for 1967 to 1985 yields approximately 400 journal references describing CDs in pharmaceutical applications. Uekama and Otagiri[12] reviewed over half of this published literature in 1986.

A search of the literature since 1986 shows that journal references have more than tripled, and patent literature has continued to grow rapidly (Fig. 1). The scientific articles have established the research applications of CDs, and patent applications reflect the increasing interest in the commercial protection of CDs in pharmaceutical products.

The commercial viability of a CD formulation was established with the marketing of 10 products, listed in Table 1. Eight products were introduced in Japan, one in Europe and Japan, and one in Europe alone. Numerous clinical trials using CD formulations have been conducted or are in progress in the United States, although currently no CD-based formulations have been approved.

More pharmaceutical products are reaching the marketplace as CD formulations and research studies exploring their applications grow exponentially. Nevertheless, the routine use of CDs in formulations is still regarded with reluctance, mainly because of uncertain regulatory acceptance of a formulation containing a nonstandard inactive ingredient.

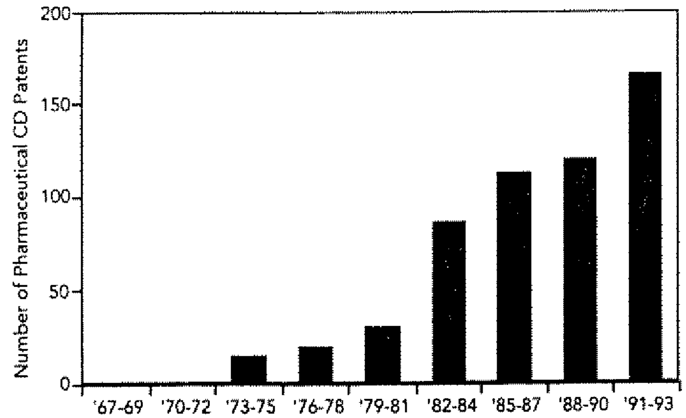


FIGURE 1. Patents and patent applications in the use of CDs in pharmaceuticals (data collected from *Chemical Abstracts* 1967–1993).

B. Scope of Review

The 6 CDs commercially available* in both quality and quantity sufficient for pharmaceutical formulations are the parent CDs (α-, ß-, and γ-CD) and 3 modified derivatives of ß-CD—methyl (M), hydroxypropyl (HP), and sulfobutylether (SBE). The objective of this review is to describe these CDs and to make it clear that, although

*1. Cerestar (formerly American Maize Products), 1100 Indianapolis Blvd., Hammond, IN 46320-1094, (800)348-9896
2. CTD, Inc., 3713 S.W. 42nd Avenue, Suite 3, Gainesville, FL 32608-2531, (904)375-6822
3. CYCLOLAB Ltd., P.O. Box 435, H-1523 Budapest, Hungary, (361) 206-51-36, 100324.1722@compuserve.com
4. CyDex L. C., 7304 West 130th Street Suite 370, Overland Park, KS 66213-2627, (913)685-8850, cydex@aol.com
5. Roquette Fréres, 4 rue Pauou, F.59022 Lille Cedex, 10.30.7797; Roquette America, P.O. Box 6647, Keokuk, IA 52632-0647 (319)524-5757; or Roquette Japan:Rhône-Poulenc Japan, Ltd., No. 16 Kowa Building Annex, 9-20 Akasaka 1-chome Minato-ku, Central P.O. Box 1649, Tokyo 107, 3585-7411
6. Janssen Biotech N. V. Drug Delivery Systems, Lammerdries 55, B-2430 Olen, Belgium, 014.22.40.15
7. Wacker-Chemie GmbH, Hanns-Seidel-Platz 4, D-8000, München 83, Germany, (089) 6279-1481; Wacker-Chemicals USA, 535 Connecticut Ave., Norwalk, CT 06854 (203)866-9400; or Wacker-Chemicals East Asia Ltd., 2-14-1, Nishi-Waseda, Shinjuku, J-Tokyo 169, (813)5272-3121
8. RingdeX, 16 rue Ballu, F 75009 Paris, France, (33) 1 40.82.35.95
9. Merican Corporation, Pharmaceuticals & Chemicals Division, 5-8, Kyobashi 1-Chome, Chuo-ku, Tokyo 104, Japan, 3-3231-3917, Fax 3-3276-0151
10. Ensuiko Sugar Refining Co., Ltd., Biobusiness Department, Sanmaruko Building 2-26-8, Nihonbashi Ningyo-cho, Chuo-ku, Tokyo 103, Japan, 3-3249-2313.

TABLE 1
Commercial Pharmaceuticals with CD-Based Formulations[306–309]

Component	Trade name	Company	Country	Formulation
PGE_1/α-CD	Prostandin Prostandin 500 Prostavasin	Ono Schwarz Pharma	Japan Germany, Italy	Intraarterial infusion
Piroxicam/ß-CyD	Brexin Cycladol	Chiesi Masterpharm	Italy Italy Belgium Netherlands Switzerland	Tablet Suppository
	Brexin	Robapharm (Pierre Fabre) Promedica	France France	Tablet
	Brexidol	Nycomed Launder	Scandanavia Germany	
PGE_2/ß-CD	Prostarmon.E	Ono	Japan	Sublingual tablet
OP-1206/α–CyD	Opalmon	Ono	Japan	Tablet
Benexate/ß-CyD	Ulgut Lonmiel	Teikoku Shionogi	Japan Japan	Capsule
Iodine/ß-CyD	Mena-Gargle	Kyushin	Japan	Gargling solution
Dexamethasone Glyteer/ß-CyD	Glymesason ointment	Fujinaga	Japan	Ointment
Nitroglycerin/ß-CyD	Nitropen	Nippon Kayaku	Japan	Sublingual tablet
Cetotiam hexetil HCl/α–CyD	Pansporin T	Takeda	Japan	Tablet
New oral Cephalosporin (ME 1207)/ß-CyD	Meiact	Meiji Seika	Japan	Tablet

CDs have no regulatory approval, a CD-based formulation faces only those hurdles to commercial development faced by other formulations.

Regulatory review of a new drug focuses on the quality, safety, and efficacy of the formulation and any excipient thereof. Therefore, we present the literature supporting the ability of CD manufactures to consistently produce defined and safe materials. We discuss the structural features that affect CDs' complexation performance to support the optimal specifications for each of the CDs. Because

the safety of the CDs is essential to their regulatory acceptance, we present *in vitro* and *in vivo* safety studies. Finally, we summarize the regulatory review process that CDs face as new excipients and the current regulatory status of the CDs in Japan, the United States, and Europe.

II. CDS AND PHARMACEUTICAL FORMULATIONS

CDs are cyclic oligosaccharides obtained from the enzymatic conversion of starch. The parent or natural CDs contain 6, 7, or 8 glucopyranose units and are referred to as alpha (α-), beta (β-), and gamma (γ-) CD, respectively. The chemical structure of β-CD (Fig. 2) shows the cyclic nature of the molecule.

Hundreds of modified CDs[13] have been prepared and shown to have research applications, but only a few of these derivatives—those containing the hydroxypropyl (HP), methyl (M), and sulfobutylether (SBE) substituents—can be used commercially as new pharmaceutical excipients. These substituents (Fig. 3) vary in size and electronic character and are attached to the CD structure through reaction with one or more of the 3 hydroxyl groups of the glucopyranose units. To evaluate the effect of substituents on the functional properties of the CDs, a general understanding of inclusion complexation is necessary.

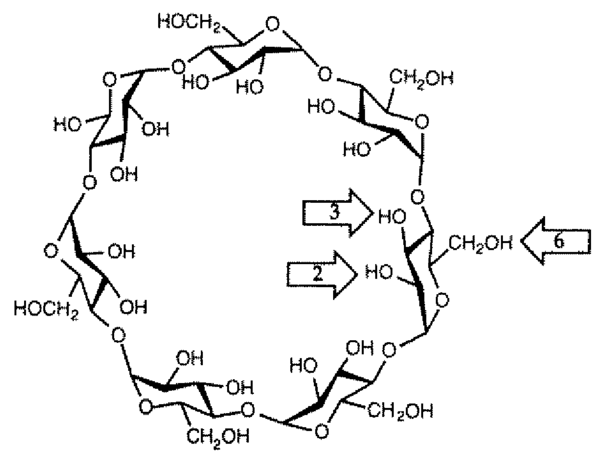


FIGURE 2. Chemical structure of β-CD. Arrows indicate the 2-, 3-, and 6- hydroxyls of a glucopyranose unit.

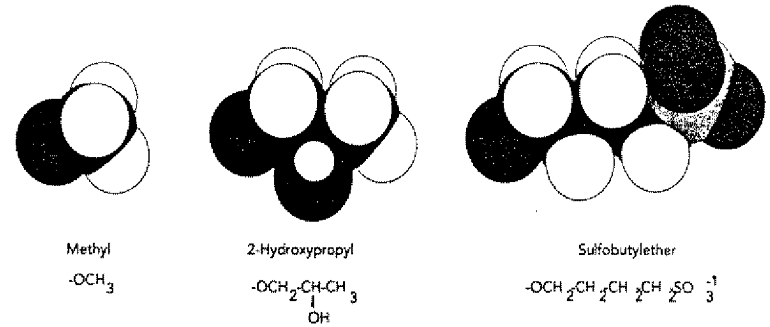


FIGURE 3. Three dimensional space-filling models showing relative sizes of methyl (M), 2-hydroxypropyl (2HP), and sulfobutylether (SBE) substituents.

A. CDs and Inclusion Complexation

1. *Complexation Equilibrium*

The 3D structure of the CD provides a cavity (Fig. 4) that is hydrophobic relative to an aqueous environment. The sequestration of hydrophobic drugs inside the cavity of the CD can improve their solubility and stability in water, the rate and extent of dissolution of the drug:CD complex, and the bioavailability of the drug when disso-

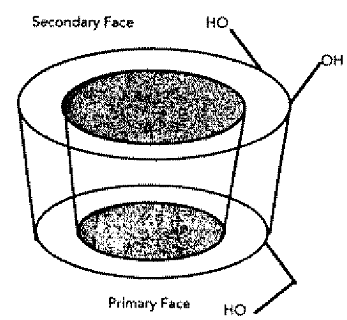


FIGURE 4. Representation of the 3D structure of the CD molecule as a segment of a hollow cone with a hydrophobic cavity and hydrophilic exterior. The secondary hydroxyls at the 2- and 3- positions exist on the secondary face of the structure, and the primary hydroxyls at the 6- position exist on the primary face.

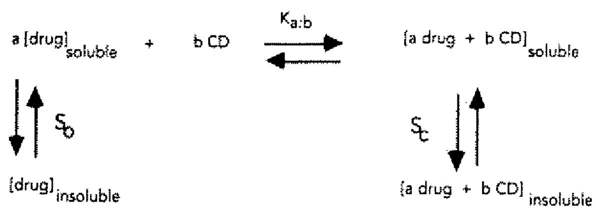


FIGURE 5. Equilibrium process describing the interaction between a CD and an insoluble drug molecule to form a soluble or insoluble complex.

lution and solubility are limiting delivery. These properties of the CD enable the creation of formulations for insoluble drugs that are otherwise difficult to formulate and deliver with more traditional excipients. Comparing the effectiveness of different CDs requires a quantitative method for contrasting their complexation properties.

CDs form inclusion complexes with hydrophobic drugs through an equilibrium process (Fig. 5) quantitatively described by an association or stability constant ($K_{a:b}$), where a and b represent the molar ratio of the sequestered drug molecule to the CD. The magnitude of this associate constant can be used to compare the effectiveness of different CDs.

Various complexes with different ratios of drug to CD molecules can be formed, depending on the type of CD used and the size and physicochemical characteristics of the drug molecule. If the drug fits into the CD cavity, a 1:1 complex results. However, if the drug is very large, several CD molecules might enclose the drug for the formation of 1:2 or higher complexes. Conversely, if the cavity is large enough, 2 drug molecules may be accommodated, resulting in a 2:1 complex. Depending on

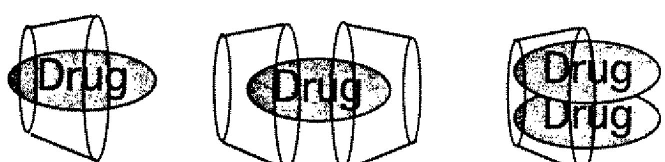


FIGURE 6. Three complex configurations: 1:1, 1:2, and 2:1.

the method used to determine the association constant, it is possible to obtain a description of the stoichiometry of the complex ($K_{a:b}$). (See Fig. 6).

Several questions are always raised by formulators considering the use of CD complexation for drug delivery. These questions can be addressed using examples from CD-based pharmaceutical products already on the market. However, this provides only an introduction into how CD complexes can be used to address various formulation problems, and this review presents additional application examples only to demonstrate the development of commercial CDs. The reader is directed to excellent reviews by Szejtli[14] and Uekama et al.[15] for extensive surveys of the application of CDs to formulation problems.

2. *Frequently Asked Questions: Drug:CD Complexes and Drug Delivery*

a. *What formulation advantages result from Drug:CD complexation?*

Improvement in solubility, dissolution, and bioavailability of drugs. CD formulations provide improved aqueous solubility to poorly soluble drugs, and the drug:CD complex often exhibits improved dissolution characteristics over other formulations of the drug. These two features improve oral bioavailability when solubility and rate of dissolution limit the availability of the drug for absorption. These factors were operative in the development of an α-CD formulation of Cefotiam hexetil hydrochloride (CTM-HE),[16,17] a broad-spectrum, semisynthetic cephalosporin antibiotic marketed in Japan as Nitropen™. Under the acidic conditions of gastric contents, CTM-HE forms a gel with poor dissolution characteristics. A variety of excipients were screened to prevent gelation, and α-CD complexation afforded the best formulation for the dissolution and solubilization of the drug.

A ß-CD formulation[18,19] of benexate hydrochloride, an antiulcer and antigastritis drug (Ulgut™ and Lonmiel™, Table 1), was also developed because of the ability of the CD complex to address solubility and dissolution problems. The classic methods used by formulators to address limited solubility and dissolution—salt formation, micronization, solid dispersions, addition of surface-active agents—were not as effective as the CD formulation. *In vivo* efficacy studies[20] demonstrated that benexate alone provided only limited inhibition of gastric ulcers (stress- and HCl-ethanol-induced), but the benexate:CD complex significantly inhibited the damage at much lower doses. The benexate:CD complex ($K_{1:1}$ = 1200 M^{-1}) showed improved dissolution (both extent and rate), which improved *in vivo* uptake in gastric tissue, providing a more efficacious formulation at a lower dose of drug.

Reduction of unpleasant side effects. Improvements in the rate and extent of dissolution of a drug can improve its rate of absorption. Reducing the contact time between the drug and the tissue mucosa can also help minimize tissue irritation. Nonsteroidal anti-inflammatory drugs (NSAIDs) cause a high incidence of gastrointestinal ulcerative lesions that are a result of both local irritation from the drug and systemic inhibition of prostaglandin synthesis by the drug. A CD formulation of piroxicam (Brexin™, Cycladol™, Brexidol™) causes fewer gastric lesions associated with the acute local tissue irritation produced by the drug alone. The protective effects of the piroxicam:ß-CD formulation ($K_{1:1}$ = 2111, 4441, and 931 M^{-1} at pH 1.2, 5.0, and 7.4, respectively[21]) have been compared to the damage caused by piroxicam alone. The damage was evaluated by endoscopic examinations[22,23] and by measuring daily and cumulative fecal blood loss.[24,25] Acute gastric lesions were significantly fewer in patients receiving the CD formulation, and there was a trend for reduction in cumulative fecal blood loss as the duration of treatment increased, suggesting that the CD formulation is more tolerable.

Improvements in drug stability. Prostaglandin E_1 (PGE_1) is marketed as an α-CD formulation in Germany and Japan under the tradenames Prostavasin™ and Prostandin™, respectively. The CD formulation was developed to increase the stability of the drug. Without the addition of a CD, PGE_1 is highly susceptible to dehydration to give PGA_1 in aqueous solution and in the solid state, but a lyophilized PGE_1:α-CD[26] provides a product with a suitable shelf life.

These examples demonstrate the application of CD complexation to a broad range of compound types. Further examples in this review will demonstrate that complexation of CDs with drugs occurs irrespective of therapeutic class. The interaction in the complex is driven by the chemical structure of the drug, its hydrophobic nature, and its ability to fit into the CD cavity.

b. Is the Drug Released from the Complex?

Because it is a reversible process, complexation of drugs by CDs improves their delivery characteristics and does not interfere with their activity. The drug is released from the complex upon dilution or by competitive displacement with endogenous lipophiles. NMR studies on Prostavasin™ (PGE_1:α-CD) diluted with infusion medium showed that the percent dissociation of the drug:CD complex is a function of dilution (Fig. 7).

The PGE_1:α-CD, however, was reported by Wiese et al.[27] to be a fairly weak association ($K_{1:1}$ ~ 900 M^{-1}). This prompts a concern that drugs with higher affinities for the CD cavity may be difficult to release by dilution. Most drug:CD complexes

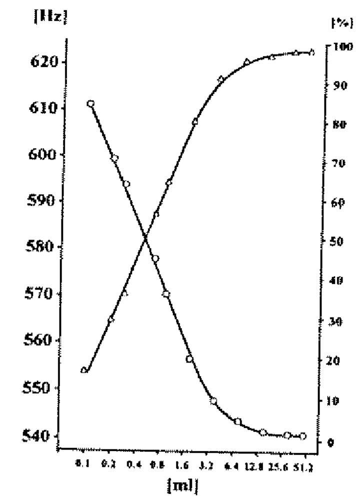


FIGURE 7. Effect of dilution on the percent dissociation of Prostavasin.[305] (Reprinted from *Pharm. Res.*, 12, 78, 1995, with permission of Plenum Publishing Corporation)

exhibit binding constants in the range of 100–20,000 M^{-1}, and Figure 8[14] demonstrates that even for the more tightly bound drugs, a 1:100 dilution will reduce the

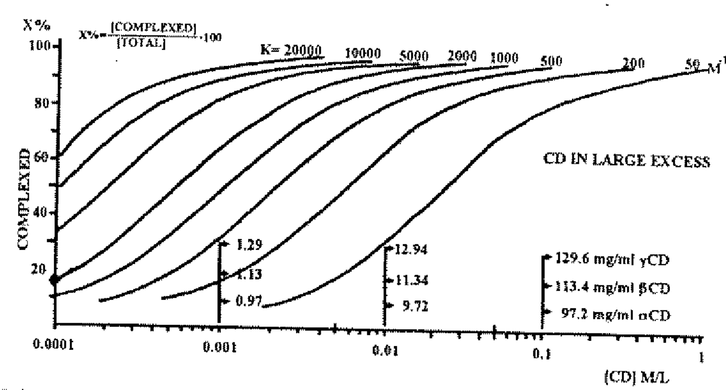


FIGURE 8. Correlation between percentage of complexed drug and CD concentration at various K values.[14] (From *Med. Res. Rev.*, 14, 1994. Reprinted by permission of John Wiley & Sons, Inc.)

percentage of drug complexed from 100% to 30%. A 1:100 dilution is readily attained upon injection or dilution in the stomach and intestinal contents.

Ophthalmic, transmucosal, and transdermal products will be the most sensitive to the strength of binding association. These routes of administration experience minimal dilution, but the drug can also be displaced from the CD cavity by competing lipophiles at the delivery site, such as triglycerides, cholesterol, bile salts, and other hydrophobic compounds, which are often in much higher concentrations.

3. Factors Affecting Complexation Performance

Complexation of drugs by CDs can be affected by the conditions of the study and by changes in the chemical structure of the CD. To follow the development of the modified CDs, we must compare differences in complexation behavior.

a. Methods for Evaluating Inclusion Complexation

Phase solubility studies typically are used to evaluate the ability of the CD to complex a drug. Higuchi and Conners[28] classified the various solubility behaviors (Fig. 9) exhibited during complex formation as A-type (a soluble inclusion compound is formed) or B-type (an inclusion compound of finite solubility is formed).

From the slope of linear portion, we can determine the equilibrium binding or association constant (K) for the 1:1 complex using the following relationship, where S_O is the intrinsic solubility of the drug under the conditions studied.

$$K_{a:b} = \frac{\text{slope}}{S_o(1 - \text{slope})}$$

Additional methods[29] are available to determine these associations or stability constants, including spectroscopy (UV,[30] F,[31] NMR,[32-34] ORD-CD[32]), potentiometry,[35-37] microcalorimetry,[33,38,39] and freezing-point depression studies.[40,41] Chromatographic methods include HPLC[42,43] and TLC[44-46] techniques.

The binding constants obtained by different methods often correlate. For example, diazepam forms a complex with ß-CD with an association constant of 220 or 208 M^{-1}, as determined by phase solubility[47] vs. circular dichroism, respectively.[48] There is a close correlation of the binding constants[49] for bendrofulazide and cyclopenthiazide, as determined by the phase solubility method (56 and 165 M^{-1}) and UV method (60 and 178 M^{-1}), respectively. The complex between dimethyl-ß-CD and hydrocortisone butyrate[50] displays binding constants of 6122 and 6039 M^{-1}, respectively, as measured by phase solubility and circular dichroism.

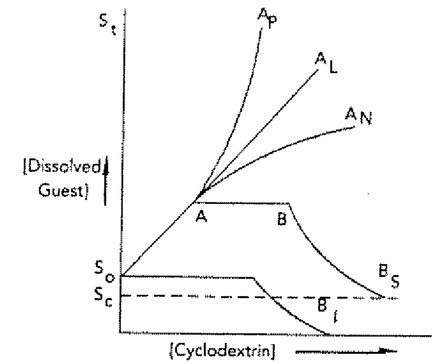


FIGURE 9. Theoretical phase solubility diagram.

However, while the above methods give similar results, the association of ß-CD and FCE245789,[36] a synthetic immunomodulator, exhibits a binding constant of 690 M^{-1} by a phase solubility determination but a binding constant over 4 times higher with a UV method. This discrepancy is due to the fact that higher-order complexes contribute to spectral changes, and these have not been accounted for in the calculation of the UV association constant. Therefore, binding constants can be used as an indicator of differences in binding only if the methods or conditions for determining the constant are equivalent or unaffected by the conditions.

pH conditions exert a unique effect on one method and not the other. Doxorubicin[51] and γ-CD form a complex with a $K_{1:1}$ of 617 and 718 M^{-1}, respectively, as measured at pH 10 by UV and circular dichroism. This close correlation was not observed when the measurements on doxorubicin were conducted at pH 7, where the binding constant for doxorubicin[52] was 235 as measured by UV but 977 M^{-1} as measured by circular dichroism. Under a given set of conditions, a drug has only 1 binding constant. Therefore, these difference reflect how the ionization state of the drug affects the analytic measurements.

In this review, comparisons of association constants have been made only if the literature studies were conducted under comparable conditions.

b. Factors Affecting Complexation Binding Constants

pH, temperature, and organic solvents can also influence the strength of a drug's binding to the CD cavity; changes in the CD structure can also influence complexation.

pH-Neutral vs. Ionic Drugs. Changing the ionization state of a drug may affect its binding to the CD. Typically, CDs bind the neutral form of a drug more effectively than the ionic form. Otero-Espinar et al.[53] reported that the binding constant for naproxen and ß-CD varied with the pH of the determination. When the drug was neutral (pH 1), the binding constant was 1379 M^{-1}, but at pH 7 the drug was completely ionized (anion) and exhibited a binding constant of only 27 M^{-1}.

Van der Houwen et al.[54] observed a similar effect in complexing mitocycin C by γ-CD. As the pH changed from 1.8 to 4.8, the binding constant increased from 48 to 249 M^{-1}. Under these conditions, the drug changed from 50% ionized (cation) to neutral. Similar results were observed for the interaction of trimethoprim[55] and HP-ß-CD, again supporting the concept that the neutral form of the drug is more readily complexed.

Temperature and solvents. Inclusion complexation is an equilibrium process, and the strength of association is affected by the temperature of the system. For example, the binding constant for the neutral naproxen molecule[53] and ß-CD decreased from 1379 to 975 to 778 M^{-1} as the temperature increased from 25°C to 35°C to 45°C, respectively. The solubility of a drug in the CD solution may increase with an increase in temperature even though the binding constant is decreasing, because the increased temperature improves the solubility of the free drug.[56,57]

Organic solvents[58-60] typically reduce the complexation of a drug in the CD by competing for the hydrophobic cavity. Recently, Loftsson et al.[61] reported on the use of water soluble polymers to increase the CD:drug complexation and improve the solubilizing effect.

CD structure: Optimal specifications for each CD. The 3 parent CDs plus the neutral—methyl (M) and hydroxypropyl (HP)—and anionic—sulfobutylether (SBE)—derivatives will be introduced separately and described in terms of their chemical identity and analytic characterization. We will ascertain optimal specifications for each CD by examining how structural variables (cavity size or substituent—type, size, number, position, polarity, or charge) affect complexing capabilities. The manufacturing variables controlling the consistent production of all of the CDs will be presented.

III. PARENT CDS AVAILABLE FOR COMMERCIAL FORMULATIONS

CDs were discovered in 1891 when Villiers[62] observed crystallization in a bacterial digest of starch. In 1903, Schardinger's[63] evaluation of the unusual crystalline dextrins suggested their cyclic nature, but their complete structural definition did not occur until the 1940s.[64,65] This coincided with identification of the enzyme responsible for their production (*Bacillus macerans amylase*, now referred to as CD glucosyltransferase: CGTase: EC 2.4.1.19) and the recognition of the complexing properties of the CD cavity. In the ensuing 30 to 40 years, extensive work resulted in the production of each of the parent CDs in bulk quantities.

The parent CDs are cyclic carbohydrates consisting of a variable number of glucopyranose units linked by 1,4-glycosidic bonds. The chemical structure of ß-CD (refer to Fig. 1) shows its cyclic nature and the 3 hydroxyl groups on each glucopyranose unit. Two of the hydroxyls are secondary alcohols and are located at the C-2 and C-3 positions of the glucopyranose unit. The third hydroxyl is a primary alcohol at the C-6 position.

The conformation of the glucopyranose units results in a 3D structure best represented by a segment of a hollow cone (refer to Fig. 4) with the secondary hydroxyls on the secondary face and the primary hydroxyls on the primary face. The hydroxyls provide the hydrophilic exterior responsible for the aqueous solubility (Table 2) of the CDs.

ß-CD has an unusually low water solubility because of the very rigid structure that results from the H-bonding of the C-2 hydroxyl of 1 glucopyranose unit with the C-3 hydroxyl of an adjacent unit.[66] In the ß-CD molecule, a complete set of 7 intramolecular H-bonds can form, effectively limiting interactions with the solvent.

TABLE 2
Physicochemical Properties of Various CDs

	α-[a]	ß-[a]	γ-[a]	2,6-DM14-ß-CD	M14-ß-CD	2,3,6-TM21-ß-CD
No. Glucose Units	6	7	8	7	7	7
Molecular Weight	972	1135	1297	1331	1340	1429
Water Solubility (gm/100mL, 25°C)	14.5	1.85	23.2	>50	>50	31
Water Solubility (Molar)	0.149	0.162	0.179	0.376	>0.373	0.217
Surface Tension (mN/m) [CD] = 0.1 w/v%		71.6		59.6	56.4	
Hydrolysis by A. Oryzae α–amylase V_{max} value (min^{-1})[310]	5.8	166	2300			
Hydrolysis Half-life (hr) 1M HCl, 60°C		5.4		12.0	2.1	

[a] Contains crystal water (wt%) of 10.2, 13.2-14.5 and 8.13-17.7 for α-, ß- and γ-CD respectively

This "belt of H-bonds" is incomplete in the other parent CDs and so allows more favorable interactions between α- and γ-CD and water molecules. This is consistent with a less favorable enthalpy and entropy of dissolution[67] for ß-CD versus α- and γ-CD. Recent studies suggest that the abnormally low water solubility of ß-CD may be exacerbated by aggregation of these rigid ß-CD molecules.[68,69] The solubility of ß-CD can be increased by disrupting this aggregation by adding solvent structure-altering substances such as urea[70-72] and inorganic salts.[73,74]

The 3D structure of the CD provides a cavity that is hydrophobic relative to an aqueous environment and that varies in size, with α-CD being the smallest and γ-CD the largest. The entrance to the cavity is wider on the secondary face than on the primary, and the cavity is nonpolar because of the presence of the glycosidic ether oxygens at O-4 and the hydrogens attached to C-3 and C-5.

The polarity of the cavity can be evaluated by fluorescence studies, although the results depend on the probe used. When 4-(N,N-dimethylamino) benzonitrile[75] was used as the probe, the cavity appeared similar in polarity to t-butyl alcohol or ethylene glycol. However, with pyrene[76] as the probe, the cavity environment appeared comparable to solvents such as n-octanoic acid, n-octanol, iso-propyl ether, and t-amyl alcohol.

A. Complexation Behavior

The properties of the parent CDs that affect their use in drug complexation are the differences in strengths of complexation resulting from differences in cavity dimensions, and their maximum aqueous solubilities. The majority of marketed pharmaceutical CD formulations have used ß-CD because of its low cost, the availability of bulk quantities, and its ability to complex numerous drug substances. From the binding constants (Table 3), it is clear that ß-CD is the most effective CD for complexing a variety of drugs.

The solubilization of a drug will be determined by the binding constant for the complex, the intrinsic solubility of the drug, and the maximum amount of CD that can be dissolved in water. Although ß-CD has the best binding characteristics for many drug molecules, it exhibits the lowest aqueous solubility. On a molar basis, α- and γ-CD are approximately 7 to 14 times more soluble than ß-CD at their maximum aqueous solubilities.

If a drug with a molecular weight of 300 gm/mole forms a 1:1 complex with each CD, and all of the CD molecules complex a drug molecule, then the maximum amount of drug dissolved at the limit of CD solubility would be 44.7, 4.8, and 52.7 mg/mL for α-, ß-, and γ-CD, respectively. Although α- and γ-CD are less effective complexing agents, they can be used at much higher concentrations, thus compensating for their weaker binding characteristics. The availability of bulk quantities of α- and γ-CD at reasonable cost should promote their increased use.

TABLE 3
Association Constants for 1:1 Complexes[a] of Various Drugs with Parent CD

Drug	Binding Constants: $K_{1:1}$ (M^{-1})		
	α–CD	ß–CD	γ–CD
Bromazepam[311]	51	77	28
Carbamezepine[312]	20	531	
2'-Carboxyl-4,4'-bis(3-methyl-2-butenyloxy) chalcone[313]	30	1900[b]	810[b]
Chlorpromazine HCl[35]	139	11,000	336
Clobazam[314]	12	58	36
Dibucaine HCl[35]	633	662	54
Digitoxin[315]		39,700[b]	16,200[b]
Diphenhydramine[39]	44	1149	
Flurbiprofen[316]	70	1966	3054
Gabapentin[317]	30	1737	268
Glibornuride[318]	30	1737	268
Hydrocortisone Butyrate[50]	282	1782	2561[b]
(RS)-2-(4-Isobutylphenyl)-propiohydroxamic acid[319]	69	14,300	160
Lorazepam[320]		928	96
Meclizine[39]	865	2238	843
Phenytoin[321]	110	850	149
Procaine HCl[35]	39	334	13
Salbutamol[322]	1.1[c]	69.3[c]	5.1[b]
Spironolactone[323]	564	24,921[b]	11,142

[a] Phase solubility studies, A_L solubility behavior
[b] B_s solubility behavior
[c] A_N solubility behavior

B. Characterization, Analysis, and Quality Manufacturing

The parent CDs are crystalline materials that can be assayed by HPLC[77] or TLC[78] for contamination by other parent CDs or linear dextrins.[79-82] Because of the lack of chromophores in their structure, the CDs are detected indirectly by postcolumn complexation[83] or directly by pulsed amperometric detection.[84] Depending on the manufacturing process, analysis of the CDs for solvent impurities and other contaminants, such as heavy metals, may be necessary. Monographs for α- and ß-CD have

been included in the USP23-NF18[85] and the *Japanese Pharmaceutical Excipients 1993* handbook.[86] Clearly the parent CDs can be manufactured and characterized as suitable for pharmaceutical use, and quality issues will not present any regulatory roadblocks.

The development of commercial quantities of the parent CDs has relied on the isolation of CGTases in high yield and with improved CD selectivity and yield. Over the past 50 years, new CGTase enzymes have been isolated from a variety of sources,[87-89] and each enzyme has been classified as an α-, ß-, or γ-CGTase, depending on the CD initially formed.

Advances in biotechnology have aided the commercial production of the CGTases used in manufacturing the CDs. The genes for many of these enzymes have been sequenced[90-94] and cloned,[94-98] which makes for economical production of the biocatalyst required for producing CDs.

Even with the availability of low-cost enzymes, manufacturing individual parent CDs requires techniques that favor the selective production of a single parent CD to the exclusion of the other two. A variety of processes for selective production have been studied, including enzymatic degradation of unwanted CDs,[99] addition of complexing additives,[100] and ultrafiltration and solid-phase techniques.

These topics, though fascinating, are beyond the scope of this review. However, more pertinent to this discussion is the result of these efforts. Enzyme yield from overexpression of the genes has lowered the cost of production, and selective manufacturing techniques have improved the isolation of each individual parent CD. As a result, a kilogram of ß-CD that cost $1,500 in 1975 can be purchased today for under $25. In addition, whereas the supply of α- and γ-CD was limited in the early 1980s, these materials are now being produced in multiton quantities at continually decreasing cost.

As we will show in Section VI, all 3 parent CDs are safe for oral delivery, but only γ-CD appears to be suitable for use in parenteral formulations. Until recently, γ-CD was not available in bulk quantities, and research laboratories explored chemically modified CDs to improve their systemic safety and aqueous solubility.

IV. DEVELOPMENT OF MODIFIED CDS FOR COMMERCIAL FORMULATIONS

Chemical modification of the parent CDs has resulted in derivatives with improved safety features. The modified CDs that are expected to have commercial pharmaceutical utility are (1) a randomly methylated derivative of ß-CD with an average molar degree of substitution (MDS) of 14, (2) 2 hydroxypropyl derivatives of ß-CD, one with an average MDS of approximately 4 and the other of 8, and (3) a sulfobutylether

derivative of ß-CD with an average MDS of 7. Glucosyl and maltosyl CDs,[101-103] which contain a mono- or disaccharide substituent, show promise for the future but will not be discussed in this review.

To discuss the modified CDs available for commercial use, the nomenclature and characterization of the derivatized CDs is presented. With this foundation, it is possible to discuss the manufacturing parameters that affect the chemical definition of the derivatives and the controls necessary to produce a defined quality derivative.

A. Nomenclature of Modified CDs

Nomenclature in the research literature is constantly evolving as new derivatives are introduced and as more is learned about the characterization of the derivatives. Hydroxypropyl derivatives have been referred to as HP-CD, 2-HP-CD, and CD-4Pr, but none of these notations adequately describe the materials. The following discussion will address the abbreviations used to distinguish substituent identity and the notations used to indicate the degree and position of substitution.

The base CD structure will be described as α-, ß-, or γ-CD, and the substituents will be noted by an abbreviation (Table 4). The average substitution level has been described in the literature as the number of substituents per single glucopyranose unit (DS) or as the number of substituents per CD molecule (MDS).

Degree of substitution may affect the properties of the CD; therefore, it is important to note which preparation was studied. The number following the abbreviation of the substituent indicates the average molar degree of substitution (MDS) rounded to the nearest whole number. For example, HP4-ß-CD indicates a ß-CD with an average of 4 hydroxyls derivatized to a hydroxypropyl substituent, but this notation does not provide any indication of the position of these substituents on the glucopyranose units.

If known, the position of the substituent on the glucopyranose unit is indicated by a number preceding the substituent abbreviation. 6-SBE1-ß-CD describes the monosubstituted sulfobutylether derivative with the substituent attached at one of the C-6- positions. Often the substituent is introduced in a random reaction process so that introduction occurs with some defined distribution at the 2-, 3-, and/or 6- positions. For these preparations, no number precedes the substituent abbreviation. HP4-ß-CD implies a tetra-substituted hydroxypropyl preparation with substituents randomly distributed over all 3 positions of the 7 glucopyranose units.

The hydroxyl group on the hydroxypropyl substituent can exist at 1 of 3 carbons. This isomeric position is noted by a number preceding the HP notation and enclosed in parentheses, for example (3HP)-ß-CD. The most commonly occurring HP derivative is the (2HP)-ß-CD, which is often referred to simply as HP-ß-CD.

TABLE 4
Nomenclature and Substituent Structures for Modified CDs

	Position of Substituent	Substituent (R) Structure	Nomenclature #[a]-XYZ[b]#[c]-CD[d]
		Parent CDs	
alpha-CD		-OH	α-CD
beta-CD		-OH	β-CD
gamma-CD		-OH	γ-CD
		Modified CDs Neutral	
Methyl Derivatives			
Dimethyl	2, 6-	-O-CH_3	2,6-DM14-CD
Methyl	random	-O-CH_3	M#-CD
Trimethyl	2, 3, 6-	-O-CH_3	2,3,6-TM-CD
Ethyl Derivatives	random	-O-CH_2-CH_3	E#-CD
Hydroxyalkyl Derivatives			
2-hydroxyethyl		-O-CH_2-CH_2OH	HE#-CD
2-hydroxypropyl	random	-O-CH_2-CHOH-CH_3	(2HP)#-CD or HP#-CD
3-hydroxypropyl	random	-O-CH_2-CH_2-CH_2OH	(3HP)#-CD
2,3-dihydroxypropyl	random	-O-CH_2-CHOH-CH_2OH	(2,3-DHP)#-CD
		Modified CDs Anionic	
Carbon-Based Derivatives			
Carboxy	6-	-CO_2M[e]	6-C#-CD
Carboxyalkyl			
Carboxymethyl	random	-O-CH_2-CO_2M	CM#-CD
Carboxyethyl	random	-O-CH_2-CH_2-CO_2M	CE#-CD
Carboxypropyl	random	-O-CH_2-CH_2-CH_2-CO_2M	CP#-CD
Carboxylmethyl ethyl	2,6-; 3-	-O-CH_2-CO_2M; -O-CH_2-CH_3	CME#-CD
Sulfur-Based Derivatives			
Sulfates	2,6-random	-O-SO_3M	S#-CD
Alkylsulfates	6-	-O-$(CH_2)_{11}$-O-SO_3M	SU#-CD
Sulfonates	6-	-SO_3M	6-SA#-CD
Alkylsulfonates			
Sulfoethylether	random	-O-$(CH_2)_2$-SO_3M	SEE#-CD
Sulfopropylether	random	-O-$(CH_2)_3$-SO_3M	SPE#-CD
Sulfobutylether	random	-O-$(CH_2)_4$-SO_3M	SBE#-CD

[a] Position of substitutents if known; if the preparation is a random distribution. then no notation implies an undefined distribution at the 2-, 3-, and 6- positions.
[b] Abbreviated notation of substituent.
[c] Average molar degree of substitution rounded to the closest whole number.
[d] Indication of parent CD structure, i.e., α-CD.
[e] M = cation

This naming system does not provide a complete chemical definition of the modified CD preparations. For example, HP4-ß-CD can represent a mixture of only tetra-substituted CD molecules or of polysubstituted CDs in which the mixture has an average substitution of 4. The notation also does not indicate the relative position of HP substituents in the substituted CD molecule. In addition, if the material is a mixture of derivatives from the mono- to hexa-substituted CDs, the name provides no representation of the percentage distribution of the substitution bands that comprise a material with an average MDS of 4.

Although the nomenclature cannot describe all the characteristics of modified CD preparations, analytic techniques and manufacturing controls have been developed to consistently produce a given composition.

B. Quality Manufacture of Modified CDs

Regulatory review of CDs focuses on the quality and safety of CD materials. The quality of a modified CD product is built into the manufacturing process design. Quality manufacturing is maintained with total quality management programs (GMP regulations, ISO 9000 standards, etc.). A product's quality is created by understanding the synthesis and work-up procedures. It is essential for building quality into the final product that we understand the requirements for raw materials and the parameters that affect the reaction and isolation procedures. Validation of the process and cleaning procedures also ensure a quality product, and this is confirmed by the analytic characterization of the product.

Because the derivatization can occur at all 3 hydroxyl positions on each glucopyranose unit, the modification reaction generates a mixture composed of a variety of polysubstituted CDs, in which the level of substitution can vary from 1 to 21 for ß-CD. Each level or band of substitution also contains a mixture of positional and regioisomers. For the mono-substituted band, 3 positional isomers exist, 2-(2HP)1-ß-CD, 3-(2HP)1-ß-CD, and 6-(2HP)1-ß-CD.

When a second substituent is introduced, the group can attach to the same or to a different glucopyranose unit. Different regioisomers are possible, as shown by AA, AB ... AD′ notation (Fig. 10). Over 100 positional and regioisomers are possible for a disubstituted derivative and close to 2 million isomers are possible for a preparation containing a distribution of substitution bands from the single mono to the fully derivatized (21 substituents) ß-CD. The heterogeneous nature of these preparations has been suggested as a factor in their improved safety relative to the parent CDs. The mixtures are amorphous and cannot crystallize because of their heterogeneous composition,[104] and for HP-ß-CD, this factor may contribute to improved renal safety over the unmodified ß-CD.

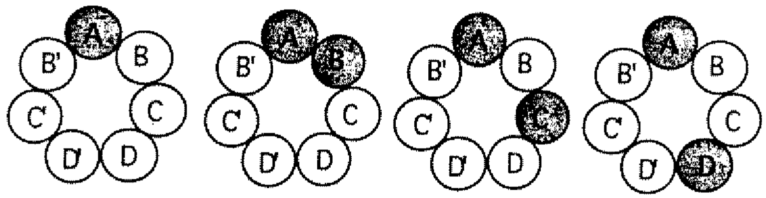


FIGURE 10. Graphic representation of the regioisomers of a disubstituted CD derivative. Structure 1 contains both substituents on the A glucose in the three possible positional compositions (2,3-; 2,6-; or 3,6-). Structures 2, 3, and 4 represent the molecules that contain a substituent on the A and B, A and C, or A and D glucose units in all possible positional compositions (2A,2B-; 2A,3B-; 2A,6B-; 3A,2B-; 3A,3B-; 3A,6B-; 6A,2B-; 6A,3B-; 6A,6B-).

The reproducibility of the composite nature of modified CD preparations is a quality issue that can be addressed by the management of the manufacturing process. The consistency of the manufacturing process and the composition of the modified CDs can be confirmed with analytic methods[10] that characterize the modified CDs and evaluate the purity of each type of preparation. Modified CD composites can be characterized by average degree of substitution, fingerprint pattern of the substitution bands, and distribution of the substituents at different regio- and positional sites.

1. Characterization of Modified CDs

a. Average Degree of Substitution

A number of methods can give us the average degree of substitution (DS or MDS) for a modified CD. Nuclear magnetic resonance (NMR) spectroscopy is the most common method; it compares the NMR signal for anomeric C-1 or its respective hydrogen to signal(s) for atom(s) distinct to the substituent. For example, methylated[105-108] and hydroxypropylated substituents[109] produce a signal for their methyl group(s) (~1 ppm), and the ratio of this signal to the anomeric H (~ 5 ppm) can be used to determine the MDS. To calculate the MDS for sulfobutylether derivatives,[110] the signal(s) for the methylene units in the butyl spacer are compared to the signal for the anomeric hydrogens.

The MDS can also be determined by additional methods. Reer and Müller[111] recently reported the use of a microcalorimetric titration for determining the degree of substitution for various methyl and hydroxypropyl CD preparations. Elemental

analysis[110] of sulfobutylether CD preparations can be used to find the degree of substitution. Each substituent contains a sulfur and a sodium atom, and the percent composition of sulfur to carbon or sodium to carbon can define the extent of substitution.

Because of the reactivity of the HP substituent's hydroxyl, further reaction of the substituent with propylene oxide generates polymerized side chains (Fig. 11). The degree of polymerization (DP) of the polypropylene glycol side chain is the ratio of the molar degree of substitution (MDS) to the degree of substitution (DS) of a glucopyranose unit.

b. Fingerprint of Substitution Bands

The average degree of substitution provides only the simplest characterization of these derivatives. Methyl-,[112] hydroxypropyl-,[113] and sulfobutylether-ß-CDs[114] are all mixtures composed of bands of different levels of substitution (Fig. 12).

Methyl-β-CDs. The methyl group is the simplest substituent used to modify the CDs and is introduced by an alkylation reaction. Alkylation can produce a wide

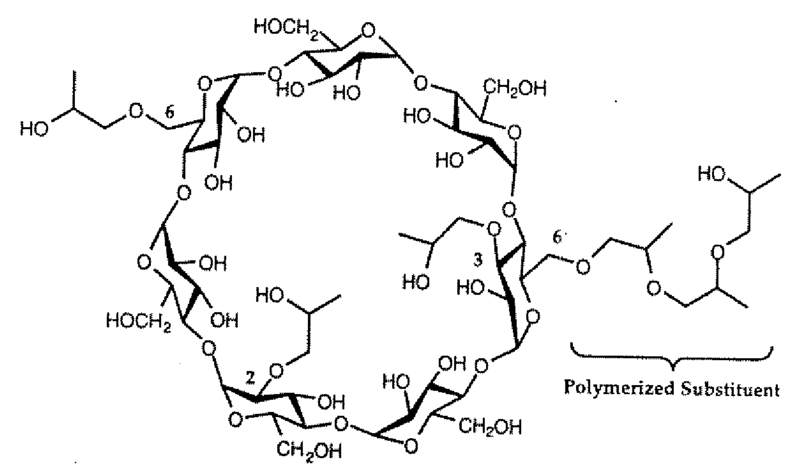


FIGURE 11. The chemical structure of one of the isomers of a tetra-substituted hydroxypropyl-ß-CD, showing substituents attached at the 2-, 3-, and 6- positions. The fourth substituent demonstrates the further polymerization of the substituent with propylene oxide.

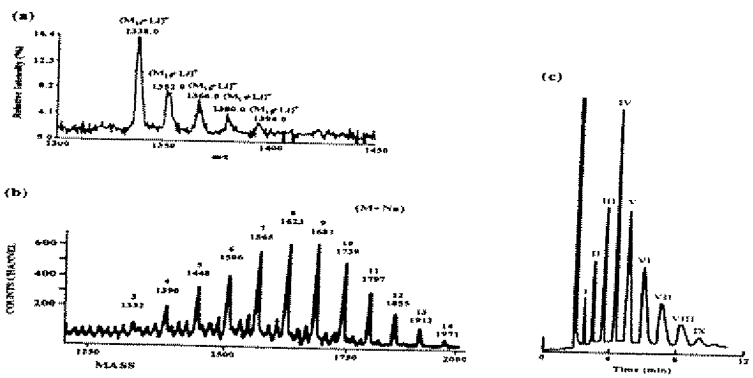


FIGURE 12. Fingerprint compositions of a) DM-ß-CD,[112] b) (2HP-ß-CD),[113] and c) SBE4-ß-CD.[114] The roman numerals in the capillary electropherogram indicate the degree of substitution for the derivatives in each peak. (Reprinted with kind permission of Elsevier Science-NL, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands)

range of products, including a fully methylated CD (2,3,6-TM-21-ß-CD), a selectively dimethylated CD modified at the 2- and 6- positions (2,6-DM14-ß-CD), or a randomly methylated product that contains, on average, 14 substituents (M14-ß-CD).

Although 2,6-dimethyl and 2,3,6-trimethyl-ß-CDs (2,6-DM14-ß-CD and 2,3,6-TM21-ß-CD) can be prepared, the materials are expensive to isolate from contamination by other alkylation products. Initial claims for the dimethylation of ß-CD[115-117] proposed that the reaction could be conducted to selectively produce a disubstituted 2,6-DM14-ß-CD, but these preparations were later shown to be mixtures.[118,119] A recent report[120] improved on the preparation of the per(2,6) alkylated CDs, but the randomly methylated product (M14-ß-CD) is likely to be the commercially available methylated CD.

The substitution pattern of these mixtures can be evaluated by mass spectrometry (MS) techniques. Various parameters affect the use of this method for quantitation. For example, Voyksner et al.[121] determined that the matrix used during negative ion fast atom bombardment-MS affects the intensity of the signals. Irie et al.[122] observed the presence of undermethylated (DS < 14) substitution bands resulting from fragmentation during MS analysis. Metzger et al.[112] determined that the addition of lithium chloride could suppress this fragmentation when the DM-CDs were analyzed by ion-spray mass spectrometry. Careful use of these MS techniques can provide an assay of the extent and fingerprint of derivatization.

Kubota et al.[119] used FAB-MS to assay the 2 major and 14 minor components isolated from commercial dimethyl-ß-CD by HPLC. By this analysis, the composition is reported as the substitution pattern on an intact CD molecule (Table 5).

A different perspective on the substitution pattern was obtained by Mischnick-Lübbecke and Krebber[123] using chemical reduction of CD derivatives followed by derivatization and analysis by gas chromatography with MS detection. The commercial dimethyl-ß-CD contained 89.9% dimethylated glucopyranose units and 10.1% trimethylated units. These values compare favorably with those of the HPLC:FAB-MS method.

Hydroxypropyl-β-CDs. The hydroxypropyl substituent is typically introduced by reaction of the CD with propylene oxide. Introduction of the hydroxypropyl substituent also produces multicomponent mixtures that can be analyzed[10] by the previously described methods. Pitha et al.[113] utilized plasma desorption MS to evaluate the degree and pattern of substitution for different preparations of hydroxypropyl-ß-CD.

Sulfobutylether-β-CDs. The reaction of butane sultone and ß-CD generates a mixture of sulfobutylether (SBE) derivatives. The presence of the anionic sulfonate substituent makes possible use of capillary electrophoresis (CE)[114] to separate the SBE-CD substitution bands and to characterize fingerprint of the compo-

TABLE 5
FAB-MS Fingerprint Composition of Commercial DM-ß-CD[119]

Type of M-ß-CD	Regioisomer Type	Percentage Mixture
DM_7		40.3
DM_6TM^a		37.7
DM_5TM_2	AD^b	3.9
DM_5TM_2	AC^b	3.4
DM_5TM_2	AB^b	7.7
$DM_4TM_3{}^c$		1.5
DM_4TM_3		5.5

[a] DM = 2,6-di-O-methyl, TM = 2,3,6-tri-O-methyl
[b] Defines the regio- position of the two trimethyl groups
[c] Contains five of the possible regioisomers

sition. The CE patterns (Fig. 13) demonstrate the ability to distinguish the fingerprints of degrees of substitution SBE1, 2, 4, and 7. The method can also distinguish compositions with the same MDS and different distributions of the substitution bands. Luna et al.[124] used anion exchange chromatography to isolate each substitution band from the mono- to deca-derivatives and characterized these by NMR and FAB-MS.

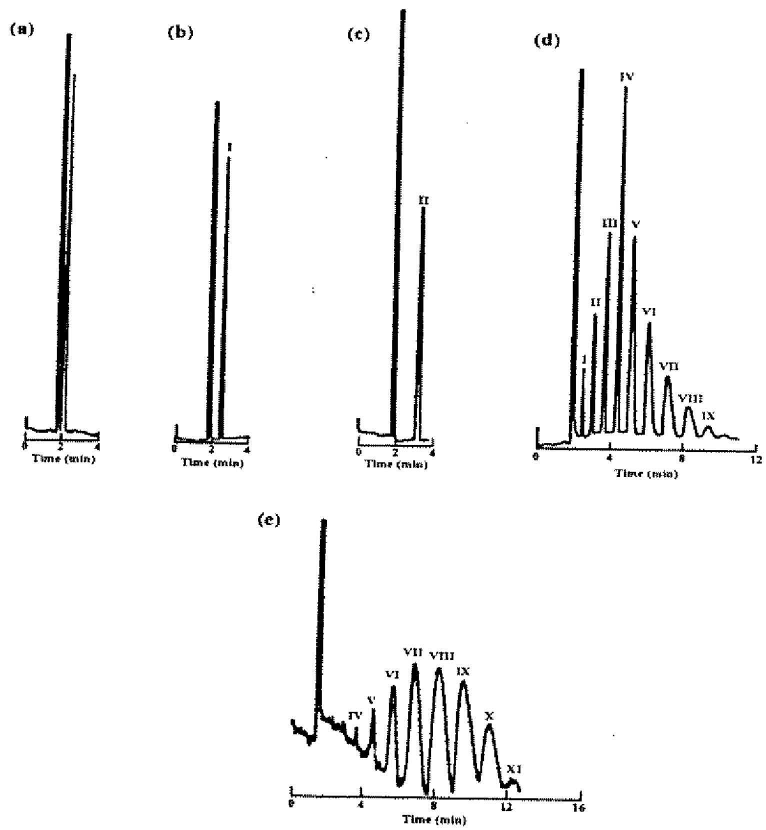


FIGURE 13. Capillary electropherograms[114] of a) ß-CD, b) SBE1-ß-CD, c) SBE2-ß-CD, d) SBE4-ß-CD, and e) SBE7-ß-CD. The roman numerals in the capillary electropherogram indicate the degree of substitution for the derivatives in each peak. (Reprinted with kind permission of Elsevier Science-NL, Sara Burgerhartstraat 25, 1005 KV Amsterdam, The Netherlands)

c. *Fingerprint of Positional and Regioisomers*

Methyl-β-CDs. Kubota et al.[119] used FAB-MS to define and quantitate regioisomers as shown for the positions of the trimethylated units at the AD and AC glucose positions, showing that 87.4% of the mixture exists as the disubstituted CD and 12.6% as permethylated CD units. Methylation of the α- and γ-CDs[125] give similar results.

Chemical reduction of the commercial dimethyl-ß-CD followed by derivatization and analysis by gas chromatography with MS detection[123] determined the percentage of 2,6- and 2,3-disubstituted versus the 2,3,6-trisubstituted units. For methylation of ß-CD, 89.3% of the modified glucopyranose units are dimethylated at the 2,6 positions and 0.6% at the 2,3 positions, and 10.1% is represented by trimethylated units.

Hydroxypropyl-β-CDs. Mischnick et al.[126] showed that reductive cleavage and methylation followed by capillary gas chromatography with MS detection can yield the extent and position of the HP-substituent. In addition, HP-ß-CD can be methylated and then hydrolyzed to the individual substituted glucopyranose units for analysis by GC/MS.[127] A comparison of these two methods (Table 6) shows the consistency of the results and the ability to distinguish an HP4-ß-CD from an HP6-ß-CD preparation.

Sulfobutylether β-CDs. The mono-substituted preparation of sulfobutylether-ß-CD (SBE1-ß-CD)[128] was shown by capillary electrophoresis to contain 3 positional isomers (Fig. 14). These individual isomers have been isolated by anion exchange chromatography, and 2D NMR techniques determined the identity of each positional isomer.

2. *Process Control Parameters: Base (pH), Temperature, Reactants*

The previous section clearly demonstrates the ability to analytically characterize CD preparations, providing manufacturers with methods to evaluate how various reaction parameters affect the composition of their products. Control of the crucial reaction parameters allows consistent production of a defined CD.

Methyl-β-CDs. The parameters that affect the reproducible production of this material have been determined. Rao and Pitha[129] showed that the percent distribution is affected by the basicity of the reaction mixture. By controlling this parameter, tem-

TABLE 6
Fingerprint Composition of 2 HP-ß-CD Preparations[126] Determined by Exhaustive Methylation & GC-MS vs. Reductive Cleavage/Methylation & GC-MS

Percentage of Each Type of Glucopyranose Unit in Different HP-ß-CD Preparations				
	HP-ß-CD: DS = 0.6		HP-ß-CD: DS = 0.9	
Type of Substituted Glucopyranose Units	Exhaustive Methylation	Reductive Cleavage	Exhaustive Methylation	Reductive Cleavage
Unsubstituted	57.9	54.4	41.2	35.7
2-monosubstituted	23.3	24.4	27.7	29.7
3-monosubstituted	4.9	4.8	5.0	6.3
6-monosubstituted	4.9	5.6	6.5	7.1
Total Monosubstituted	33.1	34.8	39.2	43.1
2,3-disubstituted	5.1	6.4	9.9	11.3
2,6-disubstituted	2.6	3.6	6.0	5.8
3,6-disubstituted	0.7	0.6	1.5	1.8
Total Disubstituted	8.4	10.6	17.4	18.9
Total Trisubstituted	0.6	0.2	2.2	2.3
Experimental DS	0.52	0.57	0.81	0.88

perature,[130] and reactant quantities, a methyl CD mixture, M14-ß-CD can be consistently manufactured.

Hydroxypropyl-β-CDs. Studies have shown that MDS and substitution patterns[104] can be modified by the type[109] and ratio of the reactants[131] and the reaction conditions. Pitha[127,132] also showed the effect of basicity on distribution of the HP-substituent. High and low concentrations of alkali favor alkylation at O-6 and O-2, respectively, and alkylation of O-2 increased reactivity at O-3. Studies have been conducted on the effect of different bases on distribution of HP at the 2-, 3-, and 6- positions.[133] With this information, process control parameters can be defined by each manufacturer to develop a validated production process for their modified HP-ß-CD.

Sulfobutylether-β-CDs. Luna et al.[134] used capillary electrophoresis to determine the effect of reactant concentrations, temperature, and base effects on the preparation of sulfobutylether derivatives. As in the other derivatizations, the ratio of the reactants and the basicity of the reaction mixture are important in determining the extent and distribution of the substituent.

3. *Purity Profile*

The purity profile of the preparations must be defined as well as the CD composition. Methods have been developed to assay for residual ß-CD content[135] in the derivatized materials. Each modified CD has a potential impurity profile that depends on the chemicals used in the process. With the application of standard assays for water content and other residual impurities, manufacturers can consistently produce a defined modified CD.

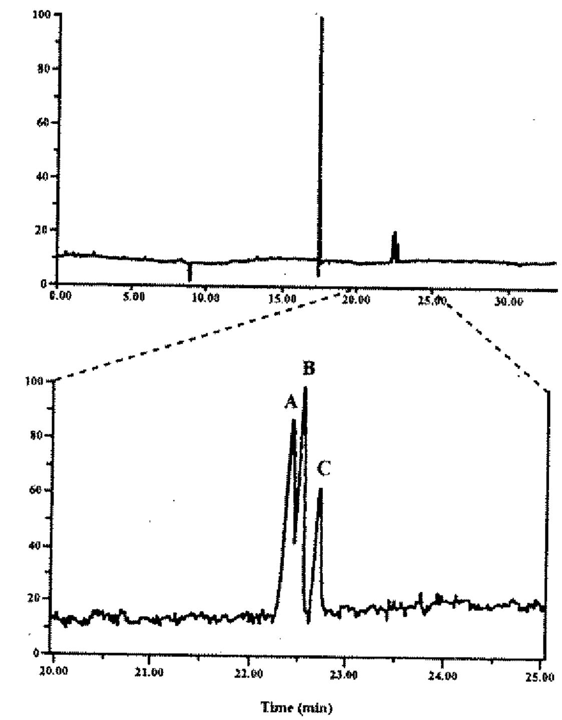


FIGURE 14. Capillary electropherogram[128] of the positional isomers in a preparation of mono-substituted SBE1-ß-CD. (Reprinted from poster presentation with permission of authors)

V. SELECTION OF IDEAL COMPOSITION FOR EACH MODIFIED CD

A. Rationale for Derivatization: The Ideal CD

The ideal modified CD is safe, manufactured at a reasonable cost, and of a quality suitable for pharmaceutical use. The water solubility and complexation characteristics of an ideal derivative will approach or exceed those exhibited by the parent CDs and should not vary significantly with changes in the degree of substitution.

The previous discussion showed that modified CDs can be characterized and reproducibly manufactured; however, defining the optimal composition for each type of derivative necessitates evaluating the effect of structural changes on the complexing ability or safety of that derivative. The commercially available modified CDs possess many characteristics of an ideal CD, in that each substituent can both increase the polarity of the structure and maintain or improve the complexation characteristics of the CD.

The CD research community has observed that introduction of substituents to the CD backbone has advantages and disadvantages. Depending on the size and nature of the substituent, derivatization at the 2- and 6- positions may extend the depth of the hydrophobic cavity (Fig. 15), but modification of the 3- and 6-hydroxyls may narrow the openings. Depending on the length and hydrophobicity of the substituent, the group may fold back into and occupy the CD cavity or may insert into the cavity of a second CD molecule.

Because the hydroxypropyl (HP), methyl (M), and sulfobutylether (SBE) substituents (refer to Fig. 3) vary in size and electronic character and can be attached to the CD backbone at all 3 positions, the optimal extent of derivatization must be determined for each modification. A balance among aqueous solubility, complexing capacity, and safety must be realized in defining optimal preparation.

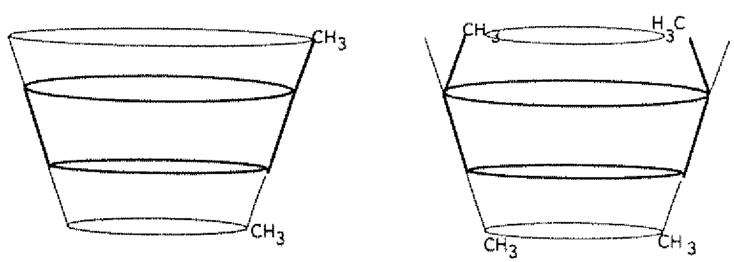


FIGURE 15. Graphic representation of the steric effects of bulky substituents at the 2- and 3- versus the 6- position of the glucopyranose units.

B. Neutral Modified CDs

1. *Methyl CD*

Methylation can be controlled to produce mono- to fully modified CDs. Introduction of the methyl substituent dramatically improves the water solubility of the derivative over that of the parent CD. Aqueous solubility increases as the number of methyl groups reaches 14 and then decreases as substitution approaches 21. As shown in Table 2, the 2,6-DM14-ß-CD and the 2,3,6-TM21-ß-CD have solubilities of 57 and 31 gm/100 ml, respectively, versus 1.8 gm/100 ml for the parent ß-CD. Introduction of the methyl groups disrupts the belt of H-bonds, effectively increasing the polarity of the derivative.

The aqueous solubility of these derivatives is adversely affected by temperature, however, and precipitation occurs during heat sterilization. The mixture of randomly methylated CD[136] (M14-ß-CD), however, exhibits a favorable water solubility (> 50 gm/mL), which increases as temperature increases.[137]

The extent of methylation is important in optimizing complexation. The introduction of the methyl substituent at the 2- and 6- positions appears to improve the inclusion of a variety of drugs to the CD cavity. The methyl groups seem to increase the hydrophobicity of the CD cavity, possibly by providing an "extension" of the cavity by introducing the nonpolar methyl groups at the 2- and 6- positions of the glucopyranose units.

More than 70% of the drugs listed in Table 7 show binding constants that are on average 5 times greater for 2,6-DM14-ß-CD than for ß-CD. Derivatization of the remaining C3 hydroxyls results in a dramatic decrease in complexing ability. For 2,3,6-TM21-ß-CD, binding strengths are only 25% of that observed for ß-CD. Permethylated CD exhibited a distorted cyclic structure,[138] and the cavity entrance may be sterically hindered by the O-3 methyl groups.[139]

The mixture of randomly methylated ß-CD, although partially modified at the 3- position, still maintains the favorable binding characteristics of 2,6-DM14-ß-CD. M14-ß-CD solubilized 26 drugs[136] more effectively than did ß-CD, and the extent of solubilization was on average 80% of that observed for the purified 2,6-DM-ß-CD preparation.

The data above suggest that an optimal definition for a commercially viable methylated CD is partially methylated ß-CD (M14-ß-CD) containing an average MDS of approximately 14 with substituents at the 2-, 3-, and 6- positions. This material is

TABLE 7
Binding Constants of Drugs Complexed with β-CD versus Methylated β-CD

Drug	$K_{1:1}$ Binding Constants (M^{-1})			Ratio Binding Constants	
	β-CD	DM-β-CD	TM-β-CD	DM-CD/ β-CD	TM-β-CD/ β-CD
Acetaminophen[324]	890	810		0.91	
Bromazepam[311]	77	227	18	2.95	0.23
Chlorambucil[325]	5250	31,400		5.98	
Clobazam[314]	58	306		5.28	
Flunitrazepam[326]	128	348	35	2.72	0.27
Fucosterol[327]	60	1610		26.83	
Flurbiprofen[328]	4340	10,060	1490	2.32	0.34
Furosemide[329]	62	160		2.58	
Hydrocortisone butyrate[50]	1782	6122		3.44	
Ibuprofen[330]	2900	9100		3.14	
Naproxen[331]	1379	26,988		19.57	
Nitrazepam[332]	131	494	64	3.77	0.49
PGA_2[333]	810	390		0.48	
PGE_2[333]	940	620	280	0.66	0.30
Salbutamol[322]	69	62		0.90	
Tolnaftate[324]	7140	17,000		2.38	

produced economically, has an aqueous solubility that increases with temperature, and has binding constants higher than those observed with unsubstituted β-CD and close to those observed with 2,6-DM14-β-CD.

2. *Hydroxypropyl CD*

Introduction of the small methyl group improved the binding characteristics of the CD if the substitution was kept to an average of 14, and at this derivatization level the material exhibited its maximum aqueous solubility. Changes in the size and type of substituent can cause the extent of substitution to affect complexation and solubility differently. Studies described below evaluated several hydroxyalkyl substituents, and the findings can help design the optimal hydroxypropyl derivative.

The smallest of the hydroxyalkyl substituents studied is the hydroxyethyl group (HE). Müller and Brauns[140] showed that increasing the molar degree of substitution from 3 to 11 decreased the solubility of hydrocortisone from 10.98 to 5.76 mg/ml for a 0.04M HE-β-CD solution (~ 5% w/v). A similar effect (Table 8) was observed for digitoxin, diazepam, and indomethacin. The decrease in solubility was thought to be caused by steric hindrance of the increased number of HE substituents; another explanation may be that as the degree of substitution (DS) increased, the degree of polymerization (DP) of the substituent increased, creating bulkier side chains that may have crowded the cavity entrance.

TABLE 8
Effect of Degree of Substitution on Drug Solubilities for Various Alkyl and Hydroxyalkyl CDs

CD (Substituent)	Drug Solubilities[140,148] (mg/mL) in 0.04 M CD Solutions[a,b]					
	Diazepam	Digoxin	Digitoxin	Hydro-cortisone	Indo-methacin[c]	Levoca-bastin
ME-β-CD ($-OCH_3$)						
MDS[c]: 6.58[d]	0.49		10.34	7.87	2.32	
MDS: 12.53[e]	0.56		11.32	8.77	2.40	
MDS: 12.6[f]	1.09		13.73	11.67	3.21	
E-β-CD ($-OCH_2CH_3$)						
MDS: 2.8	0.68			10.89	1.70	
HE-β-CD ($-OCH_2CH_2OH$)						
MDS: 3.01	0.47		9.66	10.98	2.44	
MDS: 10.71	0.27		2.20	5.76	1.62	
(2HP)-β-CD ($-OCH_2CHOHCH_3$)						
MDS: 2.03	0.36	13.17	8.09	9.55	3.48	1.10
MDS: 4.83	0.38	7.26	5.12	7.63	4.61	0.26
MDS: 8.47	0.21	4.84	3.09	4.72	3.90	0.04

[a] Literature solubilities were normalized to 0.04M [CD] in order to account for molecular weight differences of different substitutents at varied degrees of substitution
[b] Phase Solubility A_L except for Indomethacin A_N
[c] pH 7.4, indomethacin in ionized state
[d] MDS is number of substitutent per CD molecule
[e] Random derivatization
[f] Derivatized at 2,6 positions

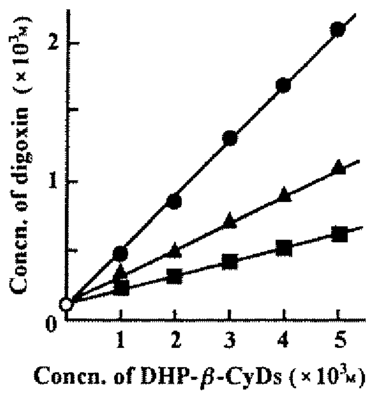


FIGURE 16. Effect of changing the degree of substitution of 2,3-dihydroxypropyl-ß-CD on the phase solubility diagrams of digoxin[141] at 25° C in water and aqueous 2,3-DHP-ß-CD solutions; solubility of digoxin O in water; ● in (2,3-DHP)-ß-CD (MDS = 2.6); ▲ in (2,3-DHP)-ß-CD (MDS = 5.9); ■ in (2,3-DHP)-ß-CD (MDS = 9.3). (Reprinted from *Chem. Pharm. Bull.*, 37, 1059, 1989, with permission of the *Chemical and Pharmaceutical Bulletin*, The Pharmaceutical Society of Japan)

There is a compromise between the steric hindrance of a substituent and its ability to extend the hydrophobic cavity. Yoshida et al.[141] showed that introduction of the (3HP) substituent (-O-CH_2-CH_2-CH_2-OH) at an MDS of ~ 6 results in higher binding constants than those observed with ß-CD, apparently because of the extension of the hydrophobic cavity. Introduction of an equivalent number of 2,3-dihydroxypropyl (2,3-DHP) substituents (-O-CH_2-CH(OH)-CH_2-OH), however, results in a decrease in binding constants. As the number of 2,3-DHP substituents increased, the solubility of digoxin decreased, as shown in the phase solubility diagrams in Figure 16. This decrease in binding was thought to be caused by steric hindrance of the larger 2,3-DHP substituent, but the 2,3-DHP substituent is also more hydrophilic than the 3HP group, and the extension of hydrophobicity of the cavity may not be realized with this substituent.

The hydroxyalkyl derivative being commercially developed is the 2-hydroxypropyl derivative of ß-CD, (2HP)-ß-CD. This often-studied derivative has been the subject of numerous clinical trials and is commercially available from several suppliers; Brandt,[142] Müller,[143,144] and Pitha[145-147] described its preparation and use.

The steric effects of the larger hydroxypropyl substituent are more pronounced than are those of the methyl group, and it appears to require a lower degree of substitution to improve binding without sterically obscuring the cavity entrance. Müller and Brauns[148] studied the effect of the degree of substitution on complexing ability (Table 9) and observed that lower degrees of hydroxypropyl substitution (DS 2–5) are more conducive to complexation. As the degree of substitution increases, the solubility of 6 different drugs decreases, but when the DS is from 4 to 8, solubility is fairly consistent.

The DS of (2HP)-ß-CD affects both the ability to form complexes and the intrinsic water solubility. Rao et al.[133] showed that increasing the degree of substitution improves aqueous solubility but impairs complexing capability. Figure 17 shows the affect of increasing the DS of (2HP)-ß-CD on its water solubility and the association constant for complexing phenolphthalein.

Lindberg et al.[149] reported that the aqueous solubility of the mono-substituted hydroxypropyl derivative of ß-CD is lower than that observed for unsubstituted ß-CD. The crystal structure of 2-(2HP)1-ß-CD[150] shows that the HP group of 1 molecule is inserted into the cavity of an adjacent CD, leading to a tightly packed crystal lattice and possibly explaining the low intrinsic solubility of HP-ß-CD with low degrees of substitution.

Controlling the degree of substitution is important in balancing water solubility and complexing capability. Two commercial preparations of (2HP)-ß-CD, Encapsin™ and Molecusol®, recognized the need for this compromise and have substitution levels that provide a balance between solubility and complexation. En-

TABLE 9
Effect of Degree of Substitution on Complexation of Drugs by HPß-CD[148]

	Solubility of Drug (mg/mL) in HP-ß-CD Solutions[a,b] at 25° C, pH 7.4			
Drug	MDS = 2.03	MDS = 4.83	MDS = 7.84	MDS = 8.47
Digoxin[a]	13.12	6.39	3.76	3.70
Digitoxin[a]	8.06	4.51	1.96	2.36
Levocabastin[b]	2.20	0.45	0.31	0.09
Indomethacin[b,c]	6.93	8.12	6.63	8.57
Hydrocortisone[b]	19.03	13.43	10.46	10.38
Diazepam[b]	0.72	0.67	0.44	0.46

[a] 5% HP-ß-CD Solution
[b] 10% HP-ß-CD Solution
[c] pH 7.4: Indomethacin in ionized state

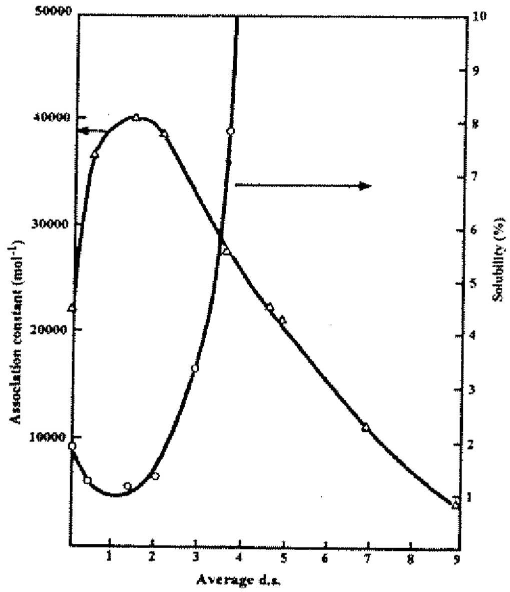


FIGURE 17. Effect of changing the degree of substitution of 2HP-ß-CD[133] on its solubility O; and the association constant with phenolphthalein Δ. (Reprinted with kind permission of Elsevier Science-NL, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands)

capsin™ and Molecusol® have MDS values of approximately 4 and 8, respectively. Although both (2HP)-ß-CD commercial preparations are unique, each manufacture can reproducibly generate materials to meet defined specifications. These (2HP)-CD derivatives appear to be equally effective in complexation and have water solubilities exceeding 50% wt/vol.

Neutral CD derivatives have been studied extensively because the prevailing attitude in the CD research community until recently was that introduction of ionic charges to the carbohydrate structure produced several disadvantages to their use as complexing agents. Müller et al.[151] indicated that anionic derivatives have limited utility because they "...exhibit weak binding forces apparently as the result of electrostatic repulsion...." Pitha[152] suggested that to be useful CD derivatives must "...retain high polarity and electroneutrality ... to sustain the lack of toxicity." Recent research shows that careful design of a charged substituent can produce a safe and effective ionic CD derivative.

C. Ionic CDs and the Selection of Sulfobutylether-ß-CD

Numerous anionic CDs have been reported (refer to Table 4). The anionic substituents are salts of carbon- and sulfur-based acids. In each family of derivatives, the charged substituent may be attached directly to the glucopyranose unit or via a neutral spacer group. These functional groups vary in size and may be introduced at different degrees of substitution. Therefore, steric and electronic factors (charge proximity and density) may effect the complexing behavior of these CDs. The effect of these structural features on complexing behavior is reviewed in order to describe the appropriate design for an ionic CD derivative.

Carboxy and Carboxyalkyl CDs. The simplest CD to contain carboxylic acid substituents (C) was reported by Casu et al.[153] with the oxidation of the primary C-6 hydroxyl groups. Yalpani and Abdel-Malik[154] described selective oxidation at C-6 to introduce the aldehyde and carboxylate functionalities. The anionic carboxylate substituent places the negative charge quite close to the carbohydrate backbone of the CD.

Parmeter et al.[155] spaced the carboxylate functionality away from the carbohydrate backbone with the preparation of carboxymethyl (CM-CD) and carboxyethyl (CE-CD) derivatives. The CM-ß-CD has subsequently been characterized.[156,157] Aqueous solubility of sodium salt of CM2-ß-CD[140] (DS = 0.26) is greater than 20 gm/100 ml, but its solubility drops as the pH of the solution decreases.

Uekama et al.[158] combined the carboxyalkyl substituent with an alkylated CD to produce carboxymethyl ethyl (CM2E11-CD) CDs. The molar degree of substitution is 1.8 for the carboxymethyl and 10.5 for the ethyl substituent. The pKa value for the carboxylmethyl group was 3.75. Above pH 6, the material is freely water soluble from ionization of the carboxyl group, but below pH 4 water solubility drops to 1.3 gm/100 ml.

The carboxylated CDs are unique in their pH-dependent solubility. Although not necessarily a desirable feature, research studies have capitalized on this feature of CME-ß-CD to provide delayed-release delivery to the intestinal tract. Delayed release of diltiazem was accomplished by complexation with CME-ß-CD.[158,159] These derivatives were also studied for their ability to slow the release of hydrophilic drugs such as theophylline[160] and for transdermal delivery of prostaglandin E_1.[161,162]

Sulfated CDs. While carboxylate derivatives have properties that vary with pH, derivatives based on sulfur acids should be unaffected by the pH of the formulation. The pK_{a1} of an alkylated sulfuric or sulfonic acid is < 1, and unlike the carboxylated CDs, the sulfated and sulfonated derivatives are always completely ionized under pH conditions typically employed in pharmaceutical preparations (i.e., pH 3–10).

Bergeron and Lee[163] introduced sulfate groups to CDs by reacting the parent CD with chlorosulfonic acid to yield the polysulfated CDs. In these derivatives, the negatively charged sulfate substituent is directly attached to the carbohydrate structure. Preparation of sulfated CDs[164] typically results in distribution of substituted species with an average MDS of 14 (S14-ß-CD).

Menger and Williams[165] spaced the sulfate functionality from the CD backbone using an 11 carbon alkyl spacer (undecyl) in the synthesis of a surfactant-like CD in which the 2- and 3- positions were converted to methyl ethers and the 6- position contained a -O-$(CH_2)_{11}$-OSO_3Na (R = SU) group.

Sulfonated and Sulfoalkylether CDs. Rajewski[110] prepared directly sulfonated CDs by introducing the sulfonic acid (SA) moiety at the C-6 position (6-SA-ß-CD). These anionically charged sulfonic acid substituents were spaced away from the CD with alkyl groups by Parmeter et al.[155] and Lammers et al.[166] in the preparation of sulfopropyl derivatives of CDs.

Stella and Rajewski[167] later described the preparation of sulfoethyl through sulfohexyl derivatives of the CDs. Sulfonate and sulfoalkylether derivatives can be prepared with different degrees of substitution,[134] are isolated as the sodium salts, and demonstrate water solubilities independent of degree of substitution.

1. Steric Effects on Complexation of Drugs with Ionic CDs

Judging from experience with the smaller methyl and hydroxypropyl substituents, steric interferences can be expected with these bulkier ionic substituents. In Sections V.B.1.-2. (beginning on page 31) we saw that full methylation of ß-CD resulted in steric hindrance to complexation because of the methyl groups "covering" the cavity opening. A more pronounced steric effect would be expected for the undecyl sulfated methyl CD described by Menger and Williams,[165] but the substituents did not seem to interfere with complexation. This highly substituted ionic CD effectively solubilizes naphthalene.

Lack of a steric hindrance by this highly substituted ionic derivative is explained through a *micellar* arrangement of the ionic substituents. The derivative was described as a micellar CD (Fig. 18) because the long hydrophobic alkyl groups in the substituent are expected to align themselves to reduce interactions with the aqueous environment similar to micelle formation. The anionic charge at the end of the alkyl chain is expected to repel adjacent substituents, effectively maintaining an opening to the CD cavity. Although the substituents are long enough to bend into the cavity, they are not expected to do so because of the hydrophilic character of the ionic sulfate, which prefers to interact with the aqueous solvent. The authors suggested that

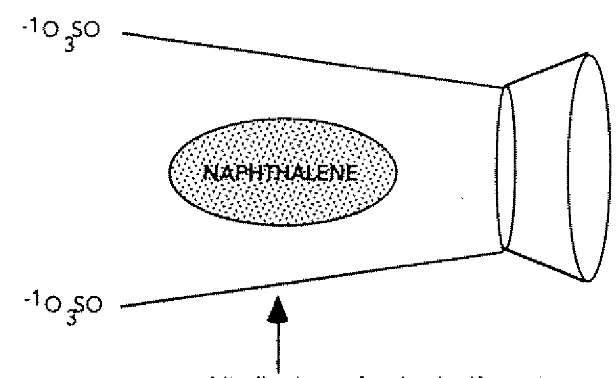


FIGURE 18. Graphic representation of the micellar arms of the undecyl sulfate substituent: The long alkyl chains align to extend the hydrophobic cavity, and the anionic sulfates repel each other, maintaining the opening of the cavity. To account for the spectral data, napthalene was proposed to interact with the micellar portion, not the CD cavity.

the interaction of napthalene may have occurred with the hydrophobic "arms" of the side chain and not with the CD cavity.

Similar structures and interactions seems to occur with the alkylsulfonate derivatives. Kano et al.[168] evaluated use of the sulfopropylether derivative of ß-CD to interact with napthalene. Higher association constants were observed for the SPE3-ß-CD (K = 2100 M^{-1}) and SPE5-ß-CD (K = 1800 M^{-1}) than for ß-CD (K = 730 M^{-1}). These higher association constants suggest that the hydrophobic propyl chains might extend the depth of the CD cavity, an effect similar to that of the undecyl sulfate substituent.

The sulfopropyl and sulfobutylether derivatives[167] have been further evaluated for their complexation with testosterone and progesterone (Fig. 19). Even though increasing the degree of substitution should produce more steric hindrance to complexation, the mono-, tetra-, and hepta-substituted sulfobutylether derivatives all displayed comparable binding abilities for the steroids, and strength of binding was similar to that observed for ß-CD. The SBE substituent behaves like the undecyl sulfate CD (refer to Fig. 18); however, complexation with SBE-ß-CDs involves the CD cavity as well as the hydrophobic butyl side arms.

Steric crowding may cause the carboxymethyl ethyl derivative (CM2E14-CD) to exhibit a lower association constant (750 M^{-1}) for diltiazem at pH 7.0 than for ß-CD (1150 M^{-1}). The lower association, however, may also be due to the proximity of the ionic charge to the CD cavity.

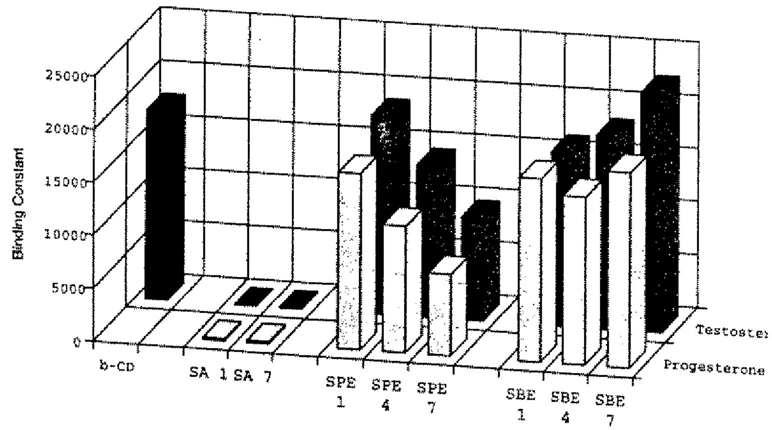


FIGURE 19. Comparison of binding constants of hydrophobic steroids,[110] testosterone, and progesterone with ß-CD and anionic CDs. SA = sulfonate anion at the 6- position, SPE = anionic sulfopropylether substituent, and SBE = anionic sulfobutylether substituent.

CD (1150 M^{-1}). The lower association, however, may also be due to the proximity of the ionic charge to the CD cavity.

Lammers[166] described the use of a less crowded carboxymethyl CD, CM7-ß-CD, to complex m-chlorobenzoic acid, but under the conditions explored (pH 1.2), both the CD and the substrate were in the neutral free-acid form. However, the di- and tetra- anions of carboxylmethyl CDs, CM2-ß-CD and CM4-ß-CD, were evaluated by Müller et al.[151] for the solubilization of digitoxin, hydrocortisone, and indomethacin.

A 10% solution of CM2-ß-CD was able to dissolve only 7.8 mg/mL of digitoxin versus the 13.5 mg/mL dissolved by a 10% solution of 2,6-DM14-ß-CD, a more sterically crowded derivative. Although the carboxymethyl group is only slightly larger than the methyl substituent, the more highly substituted dimethyl CD was the more effective solubilizing agent. This suggests that electronic, not steric, effects may limit complexing by anionic CM-ß-CD. Similar results were observed for the tetra-anion. CM4-ß-CD dissolved only 12.1 mg/mL hydrocortisone, compared to the 23.3 mg/mL dissolved by a 10% solution of 2,6-DM14-ß-CD. These results suggest that ionic interactions, not steric effects, play the major role in the reduced complexing ability.

All these studies show that it is possible to complex drugs with ionic CDs, but that complexation might be affected by the position of the charge on the substituent. In the first examples, the charge on the substituent is separated by at least 1 carbon unit from the glucopyranose units of the CD. Section 2 will describe the effects of changing the location of the charge.

2. Electronic Effects on Complexation of Drugs with Ionic CDs

a. Effect of Proximity of Charge to CD Cavity

Ionic derivatives with charges closest to the CD cavity are the carboxylate, sulfate, and sulfonate derivatives. The complexation characteristics of the directly carboxylated CDs, C-ß-CDs, have not been reported, but the highly anionic sulfated CD derivative (S14-ß-CD) does not appear to form inclusion complexes.[169] This may be due either to steric effects from the 14 sulfate substituents or to the ionic state of the CD.

The effects of charge proximity on CD complexation behavior were evaluated (Fig. 19) by studying complexation of 2 steroids by the sulfonate, sulfopropyl (SPE), and sulfobutyl (SBE) derivatives.[167] Electronic effects seem to be more of a factor than steric effects, because even when only 1 sulfonate substituent is attached at the 6- position (6-SA1-ß-CD), the derivative loses its complexing capability. The binding constant for testosterone is only 64 M^{-1} for 6-SA1-ß-CD, versus 17,800 M^{-1} for the neutral ß-CD. The attachment of a single negative charge close to the CD cavity appears to disrupt the thermodynamics driving the complexation.

When 1 sulfonate ion (SA1) is directly attached to the CD, there is minimal binding of the steroids, but as the charge is spaced away by a 3-carbon propyl (SPE1) or a 4-carbon butyl group (SBE1), the derivatives regain the binding capability of the ß-CD molecule. The mono-substituted sulfopropyl and sulfobutyl derivatives (SPE1 and SBE1) are able to bind progesterone and testosterone as well as ß-CD. This suggests that ionic substituents too close to the CD cavity disrupt the thermodynamics driving the inclusion complexation. Moving the charge away from the cavity reestablishes the complexation characteristics, but this depends on the charge density in the structure.

b. Effect of Charge Density

As the charge density increases in the sulfopropyl family from a mono- to a tetra- and hepta-anion, binding of the steroids decreases. However, when the sulfonate anion was spaced 4 methylene units away, the charge density did not adversely affect binding of the steroids. The mono-, tetra-, and hepta-substituted sulfobutylether derivatives all displayed comparable binding abilities for the steroids, and strength of binding was similar to that observed for ß-CD.

TABLE 10
Effect of Charge State[43] of Drug on Binding to Neutral ß-CD and Anionic Carboxylmethyl-ß-CD

Drug	Charge State of Drug	ß-CD (Neutral) Binding Constant (M^{-1})	CM3-ß-CD (Anionic) Binding Constant (M^{-1})
Hydrocortisone	Neutral	6200	4600
Indomethacin	Anionic	620	250
Warfarin	Anionic	520	150
Propranolol	Cationic	220	400

3. Effect of Charge State of CD and Drug on Complexation

In Section II.A.3.b. (see page 13), complexing drugs by neutral CDs (α-, ß-, γ-CD, M-ß-CD, and HP-ß-CD) was shown to be most effective with the neutral form of a drug. From these results, it is logical to question how complexing neutral and charged drugs will be affected by the charge state of the CD.

a. Anionic CDs and Neutral Drugs

The previous section has shown that ionic CDs can complex neutral hydrophobic drugs if the ionic charge is not directly attached to the carbohydrate backbone of the CD. The tri-anion of CM3-ß-CD[43] can complex hydrocortisone, a neutral drug, with an association constant 74% of that observed for neutral ß-CD (Table 10). Although this anionic derivative is less effective than the neutral ß-CD, a more favorable situation has been observed for the interaction of anionic SBE-ß-CDs and neutral drugs.

Okimoto et al.[170] reported that the anionic SBE-ß-CD (Table 11) often exhibits a 1:1 binding constant with neutral drugs that are comparable to or better than those observed for the neutral HP-ß-CD. The better binding may be due to the butyl micellar arms extending the hydrophobic cavity of the CD.

b. Anionic CDs and Ionic Drugs

When the drug and the CD are both charged, electrostatic effects may occur. Adverse electronic effects were observed for complexation between the anionic form of indomethacin and the di- and tetra-anions of carboxymethyl-ß-CD, CM2-ß-CD,

and CM4-ß-CD.[151] At pH 6.6, indomethacin is an anion, and under these conditions the anionic carboxymethyl CDs did not complex the drug at all, probably because of electrostatic repulsions. However, the tri-anion CM3-ß-CD[44] complexed the anionic forms of warfarin and indomethacin (Table 10), although only at 71% and 60% of the binding observed for neutral ß-CD.

Experience with carboxymethyl derivatives that suggests the position of the charge in the drug structure may affect the interaction with an anionic CD. Spacing

TABLE 11
Effect of Charge State of Drug on (1:1) Binding to Neutral HP-ß-CD and Anionic SBE-ß-CD

	Neutral Drug Binding Constant[a] (M^{-1})		Anionic Drug Binding Constant[a] (M^{-1})		Cationic Drug Binding Constant[a] (M^{-1})	
Drug	HP-ß-CD	SBE-ß-CD	HP-ß-CD	SBE-ß-CD	HP-ß-CD	SBE-ß-CD
Cinnarizine[b] [170]	22,500	69,700			4,000	17,500
Cinnarizine (1:2)[b] [170]	494	-			6	-
Danazol[c] [336]	76,600	94,900				
Digoxin[d] [110]	4,900	6,880				
Hydrocortisone[d] [110]	1,340	2,150				
Indomethacin[b] [170]	1,590	4,710	955	819		
Kynostatin[e] [171]	95	292			20	96
Kynostatin (1:2)[e] [171]	26	4			3	0
Miconazole[b] [170]	104,000	417,000			42,300	410,000
Miconazole (1:2)[b] [170]	45	12			11	<1
Naproxen[b] [170]	1,670	3,600	331	432		
Papaverine[b] [170]	337	1000			17	94
Phenytoin[d] [110]	1,070	756				
Progesterone[d] [110]	11,200	18,300				
Testosterone[d] [110]	11,600	22,500				
Thiabendazole[b] [170]	136	443			7	56
Warfarin[b] [170]	2,540	10,100	509	262		

[a] Binding Constants for (1:1) Complexation unless noted.
[b] HP = Encapsin™ DS = 3.5; SBE-ß-CD DS = 7
[c] HP = Roquette DS = not reported; SBE-ß-CD DS = 7
[d] HP = Molecusol® DS = 7–8; SBE-ß-CD DS = 7
[e] HP = Molecusol® DS = 7–8; SBE-ß-CD DS = 4

the charge by the butyl group in the SBE substituent appears to lessen the repulsive effects observed for shorter carboxymethyl substituent. The binding constants between the anionic forms of indomethacin and naproxen and the anionic SBE-ß-CD (Table 11) are almost equivalent to those observed for the neutral HP-ß-CD. The binding constant between the anionic warfarin molecule and SBE-ß-CD, however, is much lower than with HP-ß-CD, suggesting that the position of the charge in the drug and how it interacts with the charge in the CD may be important.

Cooperative electrostatic interaction has occurred between cationic drugs and anionic CDs. Enhanced complexation is observed when complexing the cationic form of propranolol with the anionic CM3-ß-CD (Table 10) and is probably due to cooperative electrostatic interactions. Similar positive interactions occur with SBE-ß-CD and the cationic forms of cinnarizine, miconazole, papaverine, and thiabendazole (Table 11).

c. *Anionic CDs and 1:2 Complexation*

One difference in the complexation performance of ionic versus neutral CDs is in the inability of the former to participate in 1:2 or 1:3 complexations (Table 11). The ionically charged CDs do not effectively form higher-order complexes, probably because of electrostatic repulsions between the first CD to sequester the drug and the incoming ionic CD. Johnson et al.[171] demonstrated the poor ability of SBE-ß-CD to form 1:2 complexes with kynostatin, a peptide mimetic with anti-HIV activity.

This repulsive effect is magnified as the charge density increases. As the charge

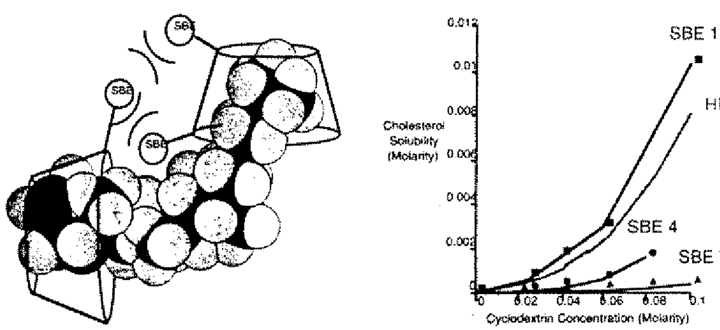


FIGURE 20. The effect of charge density[337] on sulfobutylether derivatives of ß-CD on the solubilization of cholesterol.

density of the SBE-ß-CD increases from 1 to 4 to 7 (Fig. 20), the solubility of cholesterol decreases.[172] Fortunately, SBE-CDs are able to complex drugs effectively at 1:1, so their inability to effectively participate in 1:2 complexes does not impose any practical disadvantages.

4. *Sulfobutylether ß-CD: An Optimal Ionic CD*

Studies on anionic CDs suggest that ionic derivatives can be effective complexing agents if the charge is spaced away from the CD cavity by neutral spacer groups. Studies on the sulfonate derivatives suggest that the best candidate to develop is a sulfobutylether derivative of ß-CD, because the material appears to effectively bind drugs with minimal disturbances caused by varying the degree of substitution.

SBE-ß-CD stabilized formulations of pilocarpine,[173] a hydrophilic drug, and benzyl guanine,[174] a very lipophilic compound. Parenteral SBE-ß-CD formulations of methylprednisolone[175] (IV) or prednisolone[176] (IM) were less irritating when injected than were cosolvent formulations (PEG400, ethanol, water) but were pharmacokinetically equivalent. The biocompatibility of SBE-ß-CD solutions was also observed for ophthalmic delivery, and SBE-ß-CD solutions of O,O′-dipropionyl-(1,4-xylxylene) bispilocarpate[177] significantly reduced irritation of the hydrophobic pilocarpine prodrug without affecting delivery. The oral bioavailability of cinnarizine[178] was dramatically increased when complexed with SBE-ß-CD.

SBE-ß-CD preparations exhibit good water solubilities and effective complexation characteristics at all levels of substitution, but a hepta-substituted preparation is the optimal specification for a commercial SBE-ß-CD derivative. This level of substitution effectively eliminates residual ß-CD in the product most economically. SBE7-ß-CD (Captisol™) has high intrinsic aqueous solubility (> 50% wt/vol) and exhibits binding capacities comparable to unsubstituted ß-CD but often better than HP-ß-CD. Its inability to form 1:2 complexes may contribute to potential safety benefits, as described in Section VI.

VI. SAFETY EVALUATION OF THE CDS

Commercial development of pharmaceutical CD products is only possible if the safety of the CD is established. One difficulty in reviewing the safety of the various CDs is that the results of many safety studies are contained in the manufacturers' confidential drug master file (DMF). We will review the safety of each of the CDs in terms of the published data, but this does not represent the full safety data available on these materials.

A. Parent CDs α-, ß-, and γ-CD

1. *Oral Administration: Absorption, Distribution, Metabolism, and Excretion*

To discuss the effects of these studies, an understanding of the absorption, distribution, metabolism, and excretion of the cyclic carbohydrates is useful. In general, the parent CDs are poorly absorbed after oral administration. Olivier et al.[179] determined that only 0.1–0.3% of the highest administered dose of ß-CD was excreted into the urine from rats fed a diet containing 5–10% ß-CD. Koizumi et al.[180] reported that ~ 2% of the doses administered in an isolated rat ileum closed-loop experiment was isolated as intact ß-CD from the mesenteric vein. These values are somewhat lower than the 4.2% observed by Szejtli et al.[181] and Gerlóczy et al.[182] in the urine of rats orally administered ^{14}C ß-CD.

Szabo et al.[183] suggested that in rats and rabbits CDs are absorbed by passive transport because the uptakes of ß-CD and DM-ß-CD were not inhibited by phloretin, an inhibitor of the active transport system for glucose. Absorption appears to be concentration dependent and to have no saturation limit.

Support for passive absorption was further provided by Irie et al.,[184] where an *in situ* recirculation perfusion technique showed that the amount of α-CD absorbed from the rat small intestine varied depending on the presence of bile salts. When the bile duct was ligated, only 0.89–3.12% of the α-CD concentration in the perfusate was absorbed. When sodium cholate was added to the perfusate, the amount absorbed increased to 15–19%. This increase could be completely inhibited with the inclusion of calcium chloride, suggesting that the absorption of α-CD from the intestine occurs through the passive paracellular pathway. Paracellular tight junctions are affected by endogenous calcium concentration, a finding corroborated when a similar increase in absorption was stimulated by the inclusion of disodium ethylenediaminetetraacetate (Na_2EDTA), a known complexing agent for calcium ions.

Passive paracellular absorption would explain the CDs' low oral bioavailability, but the *in vivo* bioavailabilities of < 4% seem to conflict with the 15–19% absorption of α-CD in the presence of a bile salt in the *in vitro* study. However, there may be less of an effect of bile salts on the *in vivo* absorption of α-CD because *in vivo* the bile salt concentrations are above their critical micellular concentrations (cmc ~ 9mM[335]). When the bile salts are involved in micelles, they may not provide the same level of calcium complexation observed in the perfusion studies, which utilized cholate concentrations (0.1 mM) well below the cmc.[185]

The fate of the parent CDs in the digestive tract differs due to resistance to hydrolysis and enzymatic degradation. Szejtli[186] reviewed enzymatic degradation of CDs.

Because of their cyclic structure, CDs are very resistant to hydrolysis by base and fairly resistant to stomach acid or amylases, the usual starch hydrolyzing enzymes.

CDs are completely resistant to ß-amylase but can be slowly hydrolyzed by α-amylases. The α-amylases in saliva can hydrolyze γ-CD,[187] although at only 1% the rate observed for hydrolysis of starch. In contrast, ß-CD does not appear to be hydrolyzed at all, even after 5 hours in the presence of salivary amylases.

Gerlóczy et. al[182] followed the exhalation of $^{14}CO_2$ from rats that had been orally administered ^{14}C ß-CD, ^{14}C-starch, or ^{14}C-glucose and determined that in each case the amount of radioactivity exhaled was 58%–64% of the dose. This indicated that, like glucose and starch, ß-CD was digested to glucose, which further metabolized to release $^{14}CO_2$. The time profile suggests that the metabolism of ß-CD occurred later than that of glucose or starch (6–8 hours vs. 1–2 hours postadministration, respectively). This time difference, and the previous observation that ß-CD is not hydrolyzed by the amylases, suggests a different type of metabolism course for ß-CD than for starch or glucose.

Mora et al.[188] determined that pancreatic amylase (hog) hydrolyzes ß-CD very slowly, with only 7% degraded during a 24-hour incubation. Gerlóczy et al.[189] found that whereas glucose is rapidly metabolized by the homogenized intestine of rats, the digestion of starch is slower, and ß-CD seems to be completely resistant to degradation. These results and the time profile for exhalation of $^{14}CO_2$ suggest that digestion of ß-CD occurs in the colon, not the intestine.

Antenucci and Palmer[190] showed that most human colonic bacterial strains can degrade α- and ß-CD and that this activity can be stimulated by as little as 2- to 4-hour exposure to the CDs. The typical 40-hour transit time through the human colon should be adequate to induce the bacterial enzymes to completely hydrolyze the CDs. Yoshimu et al.[191] reported that daily consumption of 10 grams of ß-CD for 2 weeks increased human fecal *Bifidobacteria* 10- to 100-fold. Suzuki and Sato[192] determined that oral digestion of ß-CD was almost complete, because only 1–4% of intact ß-CD was excreted in the feces of rats 60 hours postadministration.

All of these studies demonstrate slow degradation of ß-CD in the intestines but more extensive degradation in the colon. Flourié et al.[193] verified this in a human study with healthy volunteers and ileostomists. Analysis of the ileal effluent collected after oral administration of ß-CD during fasting (10 gm) and after eating (10 gm/meal), showed that 91% and 97%, respectively, of the ß-CD was recovered from the intestinal contents. However, when the same dose was administered to healthy volunteers, only traces of ß-CD were recovered in the feces. Colonic bacteria hydrolyzed the ß-CD with minimal hydrogen production. Approximately 1% of the ingested ß-CD was excreted in the urine as intact ß-CD, which is consistent with the oral bioavailabilities reported for ß-CD in the animal studies (0.3–4%).

As with ß-CD, little γ-CD is absorbed on oral administration. Twenty-four hours after radiolabelled γ-CD was orally administered to rats (200 mg/kg body wt) only 2% of the radioactivity was excreted in the urine and 5% in the feces. Fifty-one percent of the radioactivity was exhaled as CO_2 within 24 hours, and rapid exhalation of CO_2 in the first 2 hours suggests that γ-CD is degraded in the upper intestinal tract. This is consistent with the ability of rats to adapt and digest γ-CD, resulting in minimal changes in the cecum. Blood analysis suggested that no intact γ-CD was absorbed on oral administration. The radioactivity remaining in the carcass after oral dosing (35% of the dose) is probably due to incorporation of degradation components of the radiolabelled glucose molecules resulting from the digested γ-CD.

To summarize, only a small amount (0.3–4%) of α-, ß-, or γ-CD is absorbed intact from oral administration. γ-CD is almost completely digested in the intestines and colon. α- and ß-CD are digested to the greatest extent in the colon, with only a small contribution from intestinal hydrolysis. Although α-CD is digested more slowly than ß-CD, colonic hydrolysis of both is almost complete.

2. *Oral Administration: General Safety*

In 1957, the first report on the oral safety of ß-CD[187] erroneously suggested that the material was unsafe. Subsequent studies by Anderson et al.[194] and Gerlóczy (in Szejtli[195]) demonstrated that α- and ß-CD produced no toxic effects when fed to rats for 30 to 90 days at 1% of the diet or at 1 and 2 gm/kg/daily. The odd, nonreproducible results of the first report were probably due to the inconsistent purity of early CD materials. Residual organic solvents have been suggested to be responsible for the source of these early adverse effects.[8]

Both rodent and nonrodent studies have been conducted on all of the parent CDs. Szejtli and Sebestyén[196] reported the parent CDs to be nontoxic at very high oral doses. Mortality was not observed even in animals treated with the highest possible oral doses. Therefore, the LD_{50} in rats was greater than 12.5, 18.8, and 8 gm/kg body wt. for α-, ß-, and γ-CD, respectively.

a. ß-CD

Safety evaluation of orally administered ß-CD[179,196-199] involved extensive hematology, blood chemistry, urinalysis, and necropsy (macro- and microscopic). No significant toxic effects were observed in any of these studies after oral administration of ß-CD to mice, rats, or dogs.

No effects were observed on growth, evidenced by consistent food and water consumption and body weight changes. Small but inconsistent differences in food consumption for rats fed the 10% ß-CD diet were observed during the first week,[179] but these ultimately had no effect on growth over the 90-day study. The inconsistency may have been the result of adapting to a diet containing a slow-digesting carbohydrate. Similar results were observed for the carbohydrate control diet containing 10% lactose. Lactose, like ß-CD, is not effectively metabolized by the small intestine of the rat.[200]

Poor digestion of ß-CD and lactose by the small intestine may also have caused the increase in the cecum weight[179] for rats fed the 5% and 10% ß-CD diets and the 10% lactose diet for 90 days. The cecum, a pouch in which the small intestine ends and the large intestine begins, was the only organ that changed weight from the ingestion of ß-CD. Cecal enlargement was a typical response by mice and rats to poorly absorbed sugars and indigestible carbohydrates[201] and was not considered to have an adverse effect; it may also have caused slightly higher incidences of intermittent diarrhea in dogs treated in the 1-year feeding study. However, this effect was sporadic, infrequent, and not dose-related.

Although no macroscopic pathologies were observed, microscopic evaluation of the tissues revealed several treatment-related changes in the kidneys and the liver from the 1-year exposure of rats to ß-CD.[199] The kidneys showed a statistically significant increase in the incidence of trace amounts of pigment in the epithelium of the cortical tubules in female rats receiving the diet mixed with 2.5% and 5% ß-CD, but this was not thought to be of any toxicologic importance.

Cellular necrosis of the liver was observed in male rats receiving the 5% ß-CD diet and in female rats receiving the 2.5% and 5% ß-CD diet. A statistically significant increase in portal inflammatory cell infiltration was observed in male rats receiving the 2.5% ß-CD diet and male and female rats receiving the 5% ß-CD diet, thought to represent a mild hepatotoxicity; this was further evidenced by increases in serum liver enzymes, glutamic-pyruvic transaminase (GPT, formerly SGPT, and now referred to as alanine transaminase ALT), glutamic-oxaloacetic transaminase (GOT, formerly SGOT, and now referred to as aspartate transaminase AST), and ornithine carbamoyl transferase (OCT).

The mechanism whereby ß-CD caused the hepatic changes is unclear. Because little ß-CD is absorbed, the cause for the increase in serum levels of these liver enzymes is not readily explained. Therefore, the question remains whether very small quantities of systemic ß-CD damage the rat liver or whether oral ß-CD exerts an indirect effect on the liver tissue. One-year exposure[199] of dogs to the 0.625%, 1.25%, and 5% ß-CD diets did not result in the kidney or liver pathologies observed in the rats. Therefore, the mild hepatotoxicity may be species related and not reflective of a general hepatotoxicity.

No changes were observed in rats or dogs for hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean cell volume (MCV), erythrocyte count (RBC), and leukocyte count (WBC) after oral administration. No effects were observed in the clotting characteristic of blood from animals receiving oral ß-CD, reinforcing the observation that little of the ß-CD is absorbed upon oral administration.

Dogs fed 5% ß-CD for 1 year exhibited increased urinary protein levels and urinary excretion of calcium; these changes were not noted in the rat study.

The minimal systemic effect of ß-CD is probably due to lack of absorption from oral administration. The only changes in blood chemistry observed during the 1-year rat study involved increases in liver enzymes discussed earlier and a minor decrease in serum triglyceride from week 26, 39, and 52 for rats fed the 5% diet. This decrease in serum triglycerides was also observed in male rats fed a 10% ß-CD diet for 90 days.[179] In the 1-year dog study,[199] minor treatment-related reductions in serum lipoprotein, cholesterol, and phospholipids were observed but were not statistically significant, were not associated with any pathologies, and were considered to be of little toxicologic significance. The 1-year studies showed that the nontoxic effect levels for oral use of ß-CD are 1.25% of the diet for rats and 5% for dogs. Considering the quantity of food consumed under these conditions, this is equivalent to approximately 760 and 1899 mg/kg/day for rats and dogs, respectively.

b. α-CD and γ-CD

α- and γ-CD show oral safety profiles similar to those of ß-CD. Acute oral dosing did not result in mortality. Ninety-day feeding studies in rats and dogs[212] consuming diets containing 0%, 1.5%, 5%, 10%, or 20% α-CD or γ-CD showed effects consistent with the consumption of a poorly digestible carbohydrate such as ß-CD and lactose.

The high-dose groups (20% α-CD and γ-CD) showed soft stools or diarrhea, but this diminished as the study progressed and the animals adapted to the diets. This effect was more pronounced for animals fed the 20% lactose diet and was observed throughout the entire study.

Rats fed the 20% α-CD diet showed increased food intake during the entire treatment period, although the 20% α-CD, γ-CD, and lactose groups all showed a decrease in food conversion efficiency. However, after adapting to the 20% γ-CD diet for 1 week, the rats could almost completely digest it. The mean body weight of rats fed the 20% α-CD and lactose diets decreased slightly from the control animals, which is consistent with replacement of dietary starch with more slowly and poorly digested carbohydrates.

Analogous to the results of the ß-CD feeding studies, there was an increase in cecal weight in rats fed the α-CD, γ-CD, and lactose diets. The effect was most pronounced for the 5% and 20% α-CD and 20% lactose diets. The 20% γ-CD diet elicited smaller increases in cecal weights, reflecting better digestibility of γ-CD.

The poor digestibility of these carbohydrates resulted in an increase in fecal weight and a concurrent increase in excretion of fecal nitrogen for the 20% α-CD and lactose groups and, to a lesser extent, for the 20% γ-CD group. This may reflect a potential decrease in absorption of nitrogen-based nutrients and may explain the decreased body weights. However, no adverse health effects were observed.

Although no histopathologies were reported for any organs, relative weight of the spleen and male adrenals increased in rats fed the 20% α-CD and 10% lactose diets. In addition, slight increases in liver weights were observed for male rats fed the 20% α-CD diet and female rats fed the 20% lactose diet. All of the effects observed for these studies were reversible. Cessation of the α-CD, γ-CD, and lactose diets and return to a control diet for 28 days resulted in reversal of the changes back to values observed with the control group. This reversibility demonstrates that the effects were probably physiological adaptations to the diet.

Treatment of dogs with the 0%, 5%, 10%, and 20% α-CD and γ-CD diets resulted in minimal effects compared to those observed in the rat study.[212] Weight gains were only decreased in the last phase of the study and only for the 20% diets. Although diarrhea occurred more frequently in dogs than in rats, dogs appeared to adapt to the CD diets better than rats. Cecal enlargement was noted in the dog study, but the increase was statistically significant only for the 20% diets and was less pronounced than in the rat study. No weight changes were observed for the liver, adrenals, kidneys, or lungs, showing the dogs' greater tolerance for oral α- and γ-CD. The lack of effect on the liver or kidney was probably due to the maximal digestion of γ-CD and lack of absorption of any intact γ-CD. Absorption and excretion of α-CD is unknown. The 20% α-CD diet produced a slight decrease in plasma triglycerides, phospholipids, total protein, and 1 liver enzyme, γ-glutamyl transferase (GGT). However, no histopathologies were noted for the rat kidneys or liver.

No hematological effects were observed for dogs treated with the α-CD or γ-CD diets. In rats, the α-CD diet produced an increase in white blood cell counts for males fed 20% α-CD and 20% lactose, but there were no changes in the lymphocyte/neutrophile ratio. Although ß-CD produced increases in urinary calcium concentrations, there were no changes in the calcium content of urine for dogs treated with the α-CD or γ-CD diets. Rats fed 20% α-CD or lactose exhibited significant increases in urinary calcium concentration, but only a slight increase when fed 20% γ-CD.

TABLE 12
Equilibrium Lipid Solubilities[202] in Parent or Hydroxyalkyl CDs Solutions

CD	Solubility of Lipids in 5% CD phosphate buffered saline at 24°C: Cholesterol	Cholesteryl Oleate	Triolein	L-α–Dipalmitoyl Phosphatidyl choline	Shingo-myelin
Saline Control	0.6	0.06	0.4	4.0	0.15
α-CD	1.5	0.07	2.5	70.0	45.00
ß-CD	30.0	0.30	1.8	8.0	2.80
γ-CD	2.5	0.10	2.8	6.0	2.20
HE5-ß-CD	16.0	0.20	1.0	9.5	0.50
(2HP)5-ß-CD	40.0	0.40	1.7	8.5	2.70
(3HP)5-ß-CD	43.0	0.40	1.3	12.0	1.80
(2,3DHP)4-ß-CD	25.0	0.30	1.4	11.0	1.30

In general, oral administration of α-, ß-, and γ-CD caused several changes reflective of adapting to a diet containing a poorly digestible carbohydrate. The changes are species dependent, with rats being more susceptible than dogs. In both cases, the effects were reversible upon cessation of treatment.

3. *Potential for Increased Elimination of Endogenous Lipophiles*

The ability of CDs to nonselectively complex hydrophobic compounds raises a concern that administration of CDs may increase elimination of lipophilic nutrients or hydrophobic components found in the intestinal tract or circulatory system. For example, CDs may be able to complex vitamins from foods or hydrophobic components found in bile, which primarily consists of bile salts, cholesterol, phosphatidyl choline, and bilirubin.

Cholesterol and bile salts are bulky steroids with molecular dimensions similar to the cavity of ß-CD (7Å in diameter and 13Å in length). Irie et al.[202] reported on the comparative ability of CDs to dissolve cholesterol, triglycerides, and phospholipids, as shown in Table 12. ß-CD effectively solubilized cholesterol, but α-CD solubilized the phospho- and sphingolipid. The binding constant for the inclusion complex between cholesterol and ß-CD is 17,000 and 34,000 M^{-1} at pH 6.4 and 10.8, respectively.[203]

As expected from the different cavity dimensions of the parent CDs, the order of complexation of 1 bile salt, tauroursodeoxycholate,[204] was ß-CD > γ-CD >>> α-CD. The strength of binding between bile salts and ß-CD varies, as shown in Table 13. Cholate and chenodeoxycholate salts account for between 60% and 90% of the total bile acids and are present in approximately equal quantities, but cholate forms a much weaker complex than does chenodeoxycholate ($K_{1:1} \approx 2400$ vs. $K_{1:1} \approx 23{,}000$ M^{-1}).[205] Tan et al.[206] used NMR techniques to explain the variation in binding constants by differences in the fit of bile salt structures into the CD cavity.

These *in vitro* results suggest that ß-CD can sequester cholesterol and certain bile salts in the small intestine, potentially resulting in an increase in their elimination, with a subsequent decrease in endogenous levels of cholesterol. However, the previously described feeding studies reported only a minimal decrease in plasma cholesterol values in the 1-year 5% ß-CD dog study, and no changes were observed in the rat studies.

However, when the dietary concentration of ß-CD was raised to 10% and 20% (equivalent to approximately 6000-12,000 mg/kg body wt based on analogy to the 1-year feeding study), Riottot et al.[207] observed decreases in plasma cholesterol levels in Syrian hamsters and genetically hypercholesterolemic Rico rats (Syrian hamsters were used because they have a gallbladder and bile acid profile similar to humans). The animals were fed diets containing 1%, 5%, 10%, or 20% ß-CD for 140

TABLE 13
Association Constants for Bile Salts and ß-CD

Steroids in Bile	Association Constants (M^{-1}): $K_{1:1}$[185] Spectral Method	$K_{1:1}$[205] Microcalorimetry	$K_{1:2}$[205] Microcalorimetry
Cholate[a,b]	1,100	2,399	
Chenodeoxycholate[a,b]		22,909	
Deoxycholate[a]	2,670	61,659	724
Lithocholate[a]			
Glycocholate	410	1,950	
Glycodeoxycholate		18,620	457
Taurocholate	406	2,630	
Taurodeoxycholate		34,673	537

[a] Main constituents of bile
[b] Constitute approximately 60–90% of total bile salts in human bile at approximately equal concentrations

days. Plasma cholesterol levels were significantly reduced for both species feed the 10% and 20% diets. Plasma triglyceride levels decreased in rats and hamsters in a dose-dependent function from 1% to 20% ß-CD diets. Similar results were reported by Levrat et al.[208] and Moundras et al.[209]

The CD diets did increase fecal elimination of cholesterol and bile salts in both species. In the hamster study, fecal excretion of bile acids increased 360% over control. Complexing bile acids with ß-CD probably prevents their reabsorption, subsequently stimulating their synthesis. In fact, the bile acid content of the gallbladder of treated hamsters was 4 times that of control animals, indicating that the ß-CD diet indirectly caused an increase in bile acid synthesis. Moundras et al.[209] observed a similar increase in bile acids in the cecal contents of rats.

When enterohepatic recirculation of bile acids is interrupted, the liver increases conversion of cholesterol to bile acids, further lowering plasma cholesterol levels. Stimulation of bile synthesis should induce the activity of 2 liver enzymes, cholesterol 7α-hydoxylase and hydroxy methyl glutaryl-CoA reductase (HMG-CoA), and Levrat et al.[208] observed increases in the activity of both of these liver microsomal enzymes in rats fed a diet of 10% ß-CD for 21 days.

Abadie et al.[210] showed that ß-CD assisted in fecal elimination of chenodeoxycholate but not cholate, which makes sense considering the difference in binding affinities for the 2 bile salts. α- and γ-CD had no effect on the elimination of bile salts, which is consistent with a lack of complexation by the small cavity of α-CD and the full digestion of γ-CD in the small intestine.

The incorporation of poorly digestible starch or dietary fiber into rat diets also significantly reduced plasma cholesterol. These hypocholesterolemic effects may be due to a decrease in endogenous cholesterol synthesis and/or indirectly to shunting endogenous cholesterol into bile acid synthesis. Both effects have been observed for feeding cholestyramine, an ion exchange resin used to complex intestinal bile salts and interrupt their enterohepatic recirculation. However, a CD effect on de-novo cholesterol synthesis is not expected, and Gerloczy et al.[169] showed that oral administration of HP-ß-CD does not affect the conversion of ^{14}C-acetic acid to ^{14}C-cholesterol.

For a perspective on the sequestration of bile salts by ß-CD, a comparison can be made with the effects of orally ingested cholestyramine. Cholestyramine is classically administered to decrease intestinal bile salts, resulting in a reduction of serum cholesterol. Cholestyramine increased fecal elimination of both chenodeoxycholate and cholate at 1/10 dose of ß-CD.

Because ß-CD can sequester steroids such as cholesterol and bile salts and increase their fecal elimination, there is concern that CDs could deplete the body of lipophilic vitamins that have similar steroidal structures. Bellringer et al.[199] measured the concentration of vitamins A, D, and E in the serum and liver of dogs fed 0.625%, 1.25% and 5% ß-CD diets for 1 year. The individual results were quite variable but

did not show any significant change in these lipophilic vitamins, although the serum Vitamin A content of female dogs treated with the 5% ß-CD diet showed a minor decrease.

The fact that vitamins were not depleted is consistent with the ability of bile salts to displace vitamins A and D3 from a CD complex. Comini et al.[211] observed that chenodeoxycholate and lithocholate could completely displace vitamins A and D3 from a ß-CD complex when present in equal quantities. In the intestinal matrix, these bile salts would be present in much higher concentrations than would vitamins and would competitively fill the CD cavities. Cholate and taurocholate, however, were less effective at displacing the vitamins, which is consistent with their lower binding affinities (Table 13) for the ß-CD molecule.

To summarize, elimination of lipophilic nutrients and intestinal cholesterol or bile salts appears to be operative only when high amounts of ß-CD are incorporated in the diet (10–20% of diet, which may correspond to 6–12 gm/kg). Even when high concentrations of ß-CD are in the diet, the main effect appears to be elimination of bile salts, which are in fairly high concentrations in the intestinal matrix. The degradation of ß-CD in the colon can release the sequestered bile salts and nutrients for reabsorption. This process does not completely prevent elimination in the feces, but the elimination of bile salts by ß-CD is 10 times less effective than by cholestyramine.

4. *Mutagenicity, Carcinogenicity, and Reproductive Safety*

a. α-, ß-, and γ-CD

We can investigate the potential for interaction with genetic material (and therefore risk of carcinogenicity) by using bacterial and mammalian gene mutation assays and chromosomal aberration assays. Parent CDs do not exhibit mutagenic behavior in any of these assays.[186,196,212]

There have been no reports of tumors in oral feeding studies or in parenteral administration with any of the parent CDs, as would be expected from the negative results in gene mutation and chromosomal aberration assays. However, because ß-CD directly injures the epithelium of renal tubules on intravenous administration, various laboratories have used IV ß-CD treatment following treatment with a known carcinogen and unilateral nephrectomy to promote renal tumor development.[213-216]

Previous oral safety studies involved male and female animals, and although minor differences were observed, parent CDs do not adversely affect either gender, and the effect of CDs on reproduction is minimal. Gergely et al.[198] orally administered 200, 400, and 600 mg/kg of ß-CD to pregnant rats on the seventh to sixteenth day

of pregnancy; no maternal effects were observed, nor were there any changes in the pups' body weight, sex ratio, resorption, or visceral/skeletal formation.

Embryotoxicity and teratogenicity studies were reported for α- and γ-CD. Several 90-day feeding studies in rats (0%, 1.5%, 5%, 10%, and 20% α- or γ-CD or 20% lactose diets) and rabbits (0%, 5%, 10%, and 20% α- or γ-CD or 20% lactose diets) were conducted, and no effects were observed for maternal health or reproduction.[212] There was a slight reduction in maternal body weight in the rabbit study (20% diets) that corresponded to a slight reduction in food consumption, but this did not adversely affect fetal weight. The only effect in the rat studies was an increase in fetal renal pelvic cavitation for the 20% α-CD and 20% lactose diet groups. These observations appear to be related to the very high concentration of these poorly digested carbohydrates in the diet.

A more extensive evaluation of reproductive and developmental safety of ß-CD was reported in a study by Barrow et al.[217] Male and female rats were fed a diet containing 0.31%, 0.62%, 1.25%, 2.5%, and 5% ß-CD over 3 generations. No treatment-related effects were observed for survival, clinical condition, mating performance, or fertility of the parents. The only statistical difference observed in parental weight gain during the premating, mating, or gestation phases was a slight decrease in female weight gain (5% ß-CD diet) during the lactation phase following the first birth. This was not observed for the lower-dose levels or in the two subsequent matings.

The only adverse effect observed during this study was a dose-related decrease in pup weight gain from birth until weaning, but this was statistically significant only for the 5% ß-CD diet during days 7–14 postpartum. This preweaning growth retardation did not result in any permanent defects, and the affected pups returned to normal weights upon weaning.

Nursing by control animals of pups from the 5% ß-CD-treated mothers eliminated the growth retardation, and the nursing of pups from control animals by mothers fed the 5% ß-CD diet elicited the effect. This suggests that the 5% ß-CD diet adversely affected lactation or the nutritional content of the milk. However, no differences in milk composition could be detected, and ß-CD was not detected in the milk.

Concerns that the ß-CD diet was causing a decrease in the absorption of lipophilic vitamins from the mothers' diets were unsupported because supplementation of Vitamins A and D to the nursing mothers did not reverse the decreased pup weight gain. The observed reduction in neonatal growth can only be due to some undetected nutritional deficiency in the milk or milk yield from nursing females fed the 5% ß-CD diet. Such a deficiency would be consistent with observed increases in fecal nitrogen from animals fed 20% α- and γ-CD diets or 20% lactose diets.

FDA guidelines for carcinogenicity studies suggest that safety studies be conducted with the highest levels possible to determine maximum tolerated doses, but care should be taken to minimize possible nutritional deficiencies.[218] Reproductive studies are even more susceptible to nutritional deficiencies than are carcinogenicity studies, and the minor effects observed may be the result of trace nutritional deficiencies. However, this preweaning growth retardation did not result in any adverse developmental effects, and the animals regained normal weights upon weaning; therefore, the no adverse effect level (NOAEL) for oral ß-CD in this study was 1.25% dietary ß-CD.

5. *Parenteral Administration: General Safety*

The most far-reaching test for safety of a new excipient such as the CDs is at the systemic level, because many routes of administration ultimately result in some minor systemic exposure.

a. *Renal Effects*

α- and β-CD. Frank et al.[219] observed an IV LD_{50} for rats of 0.788 g/kg for ß-CD and 1.00 g/kg for α-CD. α- and ß-CD caused necrosis of the proximal kidney tubules (nephrosis) upon intravenous and subcutaneous administration. The proximal tubule section of the kidney functions in the reabsorption of nutrients from the glomerular filtrate. Tubule cells use a vacuole mechanism for recovery of proteins and other substances and active transporters for recovery of glucose and other nutrients. Materials in the vacuoles are degraded after fusing with lysozomes. α- and ß-CD appear to dramatically disrupt this process.

Although nephrosis did not occur in rats given 1, 2, 4, or 7 daily SC injections of 100 mg/kg α-CD, a single dose of 1000 mg/kg α-CD did result in nephrotoxicity, and repeated injections increased the damage. SC injection of 225 mg/kg ß-CD for 4 days produced lesions in only 1 of 4 rats, but daily injection of 450 mg/kg ß-CD produced severe nephrosis without any mortality.

The cause of toxicity is not clearly understood, but an order of events has been described by Frank et al.[219] Figure 21 presents diagrams of the histopathological changes in renal tubules produced by various CDs. Twenty-four hours after a subcutaneous injection of 1 g/kg α-CD or 0.67 g/kg ß-CD, light and electron microscopy of the kidney tissue revealed the presence of apical vacuoles and lysozomes in the epithelial cells of the proximal tubules. An increase in apical vacuoles has been observed as an adaptive response to the excretion of high concentrations of other osmotic agents such as

Intramuscular Injections in Rabbits

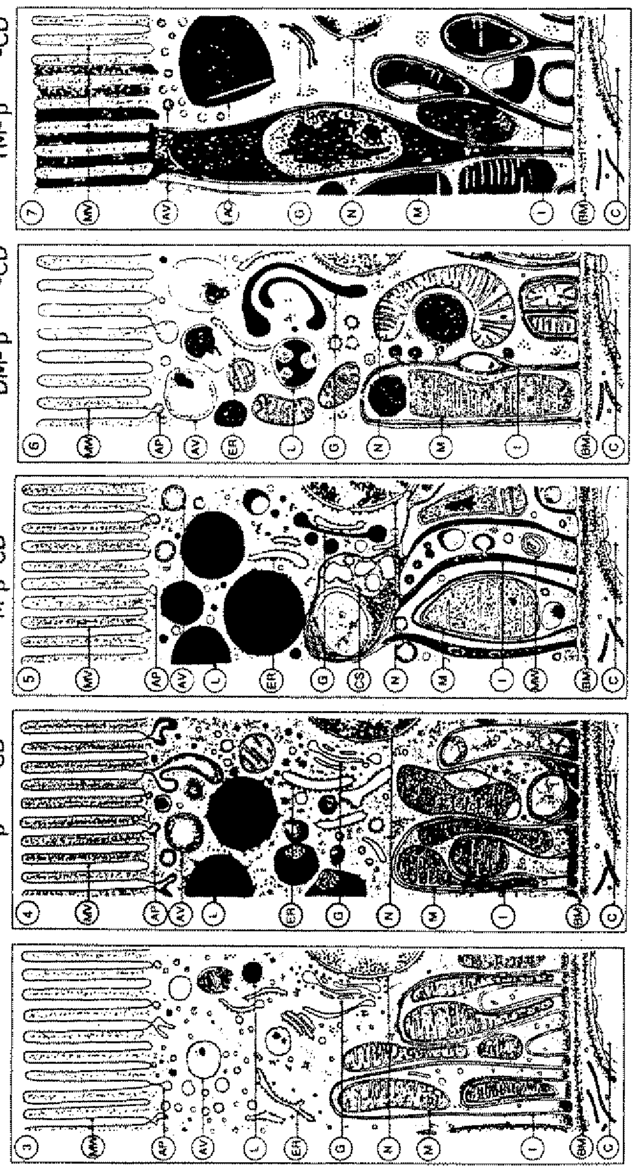


FIGURE 21. Histological representations[247] of the fine structure of the epithelial cell (x 40,000) of the proximal tubule in the rabbit treated for 12 days with IM injections of (a) saline, (b) ß-CD 50 mg/kg, (c) M-ß-CD 50 mg/kg, (d) DM-ß-CD 50 mg/kg, and (e) TM-ß-CD 50 mg/kg.
Abbreviations: MV = microvillus, AP = apical pit, AP = apical vesicle, L = lysozome, ER = endoplasmic reticulum, G = Golgi apparatus, N = nucleus, M = mitochondria, I = interstice, BM = basal membrane, and C = capillary lumen. (Reprinted with permission of Kluwer Academic Publishers)

glucose, mannitol, and dextran.[220-223] These effects were reversible upon cessation of treatment with the osmotic agent. The α- and ß-CDs, however, appear to cause additional cellular changes that are not reversible and are ultimately toxic to the cell.

α- and ß-CD treatments resulted in lysosomal structures that were often deformed by acicular (needle-like) crystals projecting through the lysosomal membranes. Both the occurrence and abundance of these microcrystals were dose dependent and were thought to be caused by precipitation of insoluble α- or ß-CD. This seems plausible for ß-CD, which has an intrinsic solubility of only 18 mg/ml, but not for α-CD, which is almost 8 times more soluble (145 mg/ml).

Two days post-injection, the proximal tubules showed extensive alterations in the vacuologenic apparatus. Apical vesicles and vacuoles were prominent at the luminal surface. Large apical vacuoles were present and showed interrupted membranes at the point of contact with adjacent lysozomes. Giant lysozomes still contained acid phosphatases but also contained long microcrystals.

Three days post-injection, large-membrane-bound vacuoles (lysozomal in origin) were observed. These vacuoles and giant lysozomes no longer exhibited an acid phosphatase content. In advance nephrosis, other organelles, such as the mitochondria and smooth endoplasmic reticulum, also showed various alterations.

Serfözö and Tóth-Jakab[224] observed less damage from intramuscular injection of 10, 20, or 50 mg/kg daily for 12 days than from a single injection of the cumulative doses, suggesting a threshold dose for irreversibility of nephrotoxicity. Hiasa et al.[225] also observed nephrotic lesions upon daily subcutaneous injection of 450 mg/kg ß-CD to rats. The treatment resulted in a decrease in body weight and an increase in average kidney weight as a percentage of total body weight. The ß-CD treatment caused such increased diuresis and urinary protein concentration that on day 7 the volume of urine and urinary protein were ~ 5 times that of control rats. The activities of succinic dehydrogenase, alkaline phosphatase, glucose-6-phosphatase, and ß-glucuronidase were all decreased in the proximal convoluted tubules. However, no changes were observed in renal acid phosphatase or nonspecific esterases.

Frijlink et al.[203] administered 500 mg/kg of ß-CD to rats and observed that 48% of the dose was excreted into the urine and 14% remained in the kidney 48 hours post-injection. Microscopic evaluation of the kidneys presented the same disrupted cellular structure described earlier. Staining the kidney sections with sulfuric iodine showed the presence of lipid in the cytoplasmic vacuoles containing the acicular (needle-like) crystals. Cholesterol formed a complex with ß-CD that precipitated as the concentration of CD increased in the solution. This B_S phase solubility behavior and the lipid staining results suggest that the microcrystals observed during nephrosis with ß-CD could be a ß-CD:cholesterol complex.

However, this explanation does not account for the crystals observed upon treatment with α-CD, which has a higher solubility than ß-CD and does not readily

form complexes with cholesterol. However, Sharma and Janis[226] showed that CDs can also precipitate lipoproteins in the order of ß- > α- > γ- > HP-ß-CD. Therefore, the microcrystals associated with α- and ß-CD nephrosis may be either CDs or CDs complexed with a variety of lipids. Whether the crystals are related to the renal toxicity of the α- and ß-CD, or how their presence disrupts cellular function and viability, is not understood.

Frijlink et al.[227] followed the pharmacokinetics of intravenous administration of 25, 100, and 200 mg/kg doses of ß-CD to rats. More than 90% of the dose was recovered unmetabolized in the urine 24 hours postadministration. Clearance for the 25 and 100 mg/kg doses was similar to that of inulin, a polysaccharide known to rapidly distribute in extracellular fluid followed by excretion at the glomerular filtration rate. Observation of nonlinear pharmacokinetics for the 200 versus the 100 mg/kg dose is consistent with the kidney-damaging effects of ß-CD at higher doses.

γ-CD. Matsuda et al.[228] administered γ-CD to mice and rats SC and IV and observed no toxic effects for doses as high as 4000 mg/kg in mice and 2400 mg/kg in rats. Schmid[229] reported that the intravenous LD_{50} for γ-CD was 10,000 mg/kg for mice and 3750 mg/kg for rats. For acute intravenous administration, γ-CD was safer than α- and ß-CD, which exhibit LD_{50} values of 1000 and 788 mg/kg, respectively.

Antlsperger[212] evaluated intravenous administration of γ-CD to rats for 30 days with daily injections of 200, 630, and 2000 mg/kg, and for 90 days with daily injections of 60, 120, and 600 mg/kg. In the 30-day treatment, several indications of adverse effects were noted for the 2000 mg/kg dose: a decrease in erythrocytes (RBC), hemoglobin, and hematocrits; an increase in reticulocyte counts; elevated plasma urea and creatinine levels; and red blood cells in the urine. Kidney damage was further observed with an increase in organ weights. Other organs affected were the spleen, liver, adrenals, and lungs, all of which increased in weight. All adverse effects were reversible in a 4-week recovery group. The 200 mg/kg dose of γ-CD for 30 days did not produce these adverse effects. The results of the 90-day study suggest that 120 mg/kg/day may be the no adverse effect level (NOAEL) for intravenous use of γ-CD.

Histologic examination of the kidney showed evidence of vacuoles in the proximal tubules. As described earlier, the vacuoles are thought to result from a change in osmotic pressure with increasing concentration of the CD requiring urinary elimination. Vacuolation is reversible upon cessation of treatment. Although vacuoles are observed during the nephrotoxic response to ß-CD, the toxic event has not been confirmed to be related to the observation of vacuoles. In the toxic response to ß-CD, acicular microcrystals are also observed, and these were not noted in the treatments with α- and γ-CD. How, why, or if these microcrystals are involved in the toxicity of ß-CD are still unanswered questions.

b. *Cytotoxicity: Hemolysis and Tissue Irritation*

Early *in vivo* administration of parent CDs showed that these compounds exhibit hemolytic activity. Irie et al.[230] reported the hemolytic effect of parent CDs on human erythrocytes and demonstrated that the damaging effect of the CDs were in the order ß-CD > α-CD > γ-CD. This cellular destruction was observed for human skin fibroblasts[231,232] and intestinal cells,[232] P388 murine leukemic cells,[233] *E. coli* bacterial cells,[234] and liposomes[235] fabricated with cholesterol and phospholipids. These *in vitro* cytotoxicity studies do not indicate *in vivo* toxicity but rather provide a method to classify CDs for their potential to destabilize or disrupt cellular membranes.

The mechanism by which the damage occurs has been studied in erythrocytes, where lysis of the red-blood cell can be easily traced by release of intracellular and membrane components. Ohtani et al.[236] used this model to try and explain CD-associated destabilization and ultimate lysis of the cellular membrane. The studies suggested that CDs extract either cholesterol (ß-CD and γ-CD) or phospholipids (α-CD) from the membrane, causing small pores. This allows leakage of potassium followed by ultimate lysis and release of heme and other intracellular components. A similar release of membrane components was observed for treatment of intestinal tissue with solutions of α-, ß-, and γ-CD[237] and in a rat nasal perfusion study[238] for DM-α-CD and DM-ß-CD solutions.

Solubilization of cholesterol by ß-CD follows Bs phase solubility behavior.[203] In the linear portion of the Bs curve, a 1:2 complex[239] is formed, but the precipitate that results at high CD concentrations is a 1:3 complex.[240,241] The stability constant for the cholesterol:ß-CD complex was 17,000 M^{-1} at pH 6.4 and 34,000 M^{-1} at pH 10.8.[203] Irie et al.[230] indicated that the stability constant for the cholesterol:α-CD complex was significantly smaller than that observed for ß- and γ-CD, which would explain the minimal extraction of cholesterol from RBC by α-CD. This also explains why increasing the cholesterol content of liposomes rendered them more resistant to destruction by α-CD.[235]

Evaluation of whole blood versus isolated erythrocytes shows that cytotoxicity of the CDs is diminished 10-fold by the presence of hydrophobic serum components. The inclusion of serum lipophiles in the CD cavity appears to limit extraction of cholesterol or other membrane components, which explains the lack of *in vivo* hemolysis except when very high doses of CDs are given intravenously.

The membrane-damaging effects of CDs are observed *in vivo* only at high concentrations; for example, intramuscular irritation of *M. vastus lateralis* rabbits injected with CD solutions[141] increased in the order of damage DM-ß-CD > α-CD > γ-CD. Svendsen[242] observed that intramuscular injections of ß-CD depleted the creatine kinase (CK) content of muscle tissue at the injection site. A decrease in CK is a biochemical marker for local muscle toxicity.

B. Dimethyl-ß-CD: A Neutral CD Derivative

Derivatization of parent CDs was performed to increase the intrinsic solubility of the CD molecule with the hope that improved solubility would eliminate renal toxicity resulting from the proposed precipitation of the parent CDs in the renal proximal tubules.

1. Oral Administration: Absorption, Distribution, Metabolism, and Excretion

Szabo et al.[183,243] evaluated the intestinal absorption of ß-CD and DM-ß-CD in rats using a ligated loop technique and observed that the amount of DM-ß-CD absorbed depended on the concentration of perfusate (1–100 mM). The absorption of DM-ß-CD or TM-α-CD and TM-ß-CD was not affected by phloretin, an inhibitor of the active transporter for glucose absorption, suggesting that these CDs are also absorbed by passive transport.

Incubation of DM-ß-CD or ß-CD with the colonic contents indicated that colonic bacteria could degrade ß-CD and DM-ß-CD. Bacterial decomposition of DM-ß-CD, however, was 4 to 8 times slower than that of ß-CD. This is consistent with the results of Szatmári and Vargay,[244] who followed the pharmacokinetics of ^{14}C-radiolabelled DM-ß-CD upon oral administration (100 or 1000 mg/kg) to rats—blood levels were very low and not statistically different, suggesting that absorption was not dose dependent, which is inconsistent with results reported earlier.[245]

DM-ß-CD was rapidly excreted unmetabolized in the feces, with 78%, 86%, and 96% cumulative excretion in 24, 48, and 72 hours. A small percentage (6.3%, 8.6%, and 9.6%) of the dose was observed in the urine during the time periods studied. With rapid urinary excretion, less than 1% of the oral dose was found in the brain, heart, lung, liver, spleen, or kidneys, indicating that DM-ß-CD does not accumulate in tissues.

These results show that DM-ß-CD is less susceptible than ß-CD to colonic digestion. DM-ß-CD and ß-CD are both rapidly cleared by the kidneys, but DM-ß-CD is absorbed to a greater extent (~ 10% versus 0.3–4%). Further studies on oral safety have not been reported, possibly because of extreme renal toxicity observed from systemic exposure to DM-ß-CD.

2. Parenteral Administration: General Safety

a. Adsorption, Distribution, Metabolism, Excretion

Szabo et al.[183] showed that 99.4% of an IM injection of DM-ß-CD (150 mg/kg) was eliminated in the urine in 24 hours, in contrast to only 10% of a similar dose of TM-

ß-CD. Similar results for DM-ß-CD were recorded by Szatmári and Vargay[244] for IV administration of 40 mg/kg ^{14}C-radiolabelled DM-ß-CD to rats. Rapid excretion of unmetabolized DM-ß-CD in the urine produced 72%, 78%, and 81% cumulative excretion in 24, 48, and 72 hours. A small percentage (~ 6%, 11%, and 14%) of the dose was observed in the feces from a small amount of biliary excretion.

Yamamoto et al.[246] administered ß-CD (50 or 150 mg/kg) and DM-ß-CD (50 mg/kg/day) to rats for 6 days and observed that the rate of renal clearance was close to the glomerular filtration rate, with ~ 85–90% urinary recovery in 6 hours. Although the DM-ß-CD was given at ⅓ the dose of ß-CD, the treatment resulted in a significantly higher increase in plasma levels of the liver enzymes glutamate pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT), indicating some hepatic disorder.

The histological damage caused by methylated CDs at doses much lower than required for ß-CD, the limited excretion of TM-ß-CD, and the increase in liver enzymes in the plasma indicate that methylated CDs are more systemically toxic than ß-CD.

b. Renal Effects

Renal nephrosis[245] was observed for methylated ß-CDs following IM injections of as little as 50 mg/kg/day over 12 days. Figure 21 (see page 58) illustrated the histological changes observed for parenteral treatment of rats with ß-CD, M-ß-CD, DM-ß-CD, and TM-ß-CD. Histological changes in the renal proximal tubules mirrors those observed for the progressive nephrosis induced by ß-CD at 450 mg/kg for 1 week or a single dose of 788 mg/kg (the LD_{50} for ß-CD).

Treatment with ß-CD and DM-ß-CD also increased blood supply of the nephron, as observed by increased dilation of the capillaries compared to controls. M-ß-CD and TM-ß-CD, however, caused a decrease in the number of open capillaries. Ultimately, all CDs caused some necrosis of the glomerular capillaries and the afferent arterioles feeding the glomerulus.

The damaging effect of the methylated CDs was in the order of TM-ß-CD > M-ß-CD > DM-ß-CD > ß-CD. The increasing damage caused by each of the methylated derivatives does not follow the order of intrinsic water solubility, which is ß-CD < M-ß-CD < TM-ß-CD < DM-ß-CD. This suggests that other characteristics of the CD must be operative in the toxicity mechanism.

c. Cytotoxicity: Hemolysis and Muscular Damage

Extensive alterations in the cellular components of the renal tubule cells by methylated CDs suggest that these derivatives are more damaging than the parent CDs. The

The membrane-damaging effect of the methylated derivatives was evaluated by *in vitro* cytotoxicity studies with erythrocytes. Jodál et al.[247] and Yoshida et al.[248] evaluated the effects of methylated CDs on human erythrocytes. DM-ß-CD was more hemolytic than was TM-ß-CD or randomly methylated M14-ß-CD. Figure 22 shows that the damaging effects are in the order of DM-ß-CD $\gg$ ß-CD > α-CD > HP-ß-CD > γ-CD. In addition to the ability to extract cholesterol from the membranes, the enhanced damaging effects are probably due to surface activity of the methylated derivatives (refer to Table 2, page 15).

3. *Methylated CDs: Permeation Enhancers?*

The increased solubility of the methylated derivatives did not reduce the renal toxicity of the CDs. High surface activity and improved complexation of methylated CDs results in extreme renal toxicity and membrane-damaging activity, which prevents their use in parenteral formulations. However, the ability of methylated CDs to destabilize cellular membranes can increase absorption of drugs by the transcellular pathway for oral, nasal, or rectal administration.

Dimethyl-ß-CD may improve the bioavailability of drugs by enhancing penetration. The high surface activity of methylated CDs can increase absorption by the transcellular pathway, which is similar to the effects observed for surfactants.[249,250]

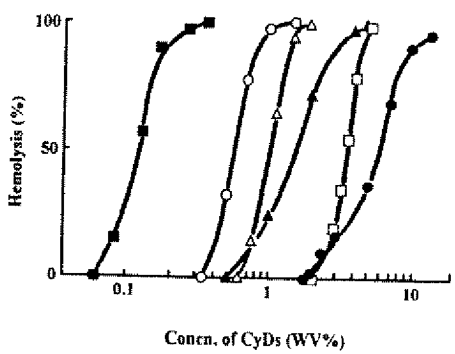


FIGURE 22. Hemolytic effects of CD derivatives on human erythrocytes[248] in isotonic phosphate buffer (pH 7.4) at 37° C for 30 mins; △ α-CD; ○ ß-CD; □ γ-CD; ■ DM-ß-CD; ▲ HP-ß-CD; ● HE-ß-CD. (Reprinted with kind permission of Elsevier Science-NL, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands)

Absorption of ketoprofen,[251] carmofor,[252] ethyl 4-biphenylacetate,[253] indomethacin,[254] 17-ß-estradiol,[255] progesterone,[256] insulin,[257] and human growth hormone[250] has been enhanced by use of the methylated CDs.

C. Hydroxypropyl-ß-CD: A Neutral CD Derivative

Although the increased solubility of methylated derivatives could not reduce the renal toxicity of the CDs, introduction of other functional groups have dramatically reduced the renal effects observed for α-, ß-, and DM-ß-CD. Hydroxypropyl and sulfobutylether derivatives have both shown excellent safety profiles.

1. *Oral Administration: Absorption, Distribution, Metabolism, and Excretion*

Gerloczy et al.[258] dosed rats with a 0.15% or a 16% solution of ^{14}C-radiolabelled HP5-ß-CD to achieve oral doses of 15 and 40 mg/kg, respectively. Three percent of the radiolabel was excreted in the urine and 71% in the feces, and 3% was exhaled as ^{14}C-CO_2. Radioactivity appeared in the blood within 5 minutes postadministration, reached a maximum at approximately 45 minutes, remained nearly constant for 3 hours, but was eliminated completely in the urine in 24 hours. There was no accumulation of radioactivity in any organs.

The reported bioavailability (3%–6%) may have been overestimated in this study because of the presence and absorption of residual radiolabelled polypropylene glycol produced during the hydroxypropylation of ß-CD. This by-product is removed in commercial production of HP-ß-CD but is difficult to remove completely on the microscale preparation of the labelled material.

Monbaliu et al.[259] observed only a 3% bioavailability for a single administration of a 20% wt/vol solution (200 mg/kg dose) of ^{14}C HP-ß-CD to dogs, and only a trace of radioactivity was absorbed for administration to rats. Eight-six percent of the administered radioactivity was excreted in the feces of both species, but only 60% was excreted as intact HP-ß-CD. Therefore, limited metabolism of HP-ß-CD does occur in the intestinal tract. There was some indication that the plasma levels of radioactivity may have been metabolites resulting from intestinal digestion of HP-ß-CD.

Low oral absorption of HP-ß-CD was also observed in humans. HP-ß-CD was undetectable in the plasma or urine of human volunteers who ingested 1 and 3 grams of HP-ß-CD.[260] In general, HP -ß-CD is more resistant to digestion than α- and ß-CDs, which are digested more slowly than γ-CD. HP-ß-CD appears to have a very low oral absorption (~ 3% or less).

2. Oral Administration: General Safety

The published literature contains limited reports on the oral safety evaluation of HP-ß-CD. Product literature from HP-ß-CD manufactures indicate that oral safety has been assessed in mice, rats, dogs, and monkey for 2-week to 1-year dosing periods. Doses reached as much as 5000 mg/kg/day. No adverse effects were noted, except for an increase in diarrhea in dogs treated with 5000 mg/kg.

3. Mutagenicity, Carcinogenicity, and Reproductive Safety

Coussement et al.[261] observed no mutagenicity for HP-ß-CD in the Ames bacterial mutation assay and no chromosomal aberrations in the mouse micronucleus assay. Similar results for the Ames assay were reported by Brewster and Bodor.[262]

Reproductive safety was evaluated for both intravenous and oral administration of HP-ß-CD to rats and rabbits.[261] A Segment II study evaluating the embryotoxicity and teratogenicity of the material showed no effects for pregnant animals from implantation through gestation (during organogenesis). No adverse effects on rat or rabbit pups were observed, even when 400 mg/kg was administered intravenously to the dams. Oral administration of up to 5000 mg/kg HP-ß-CD to pregnant rats produced no maternal toxicity, embryotoxicity, or teratogenicity. Oral administration of 1000 mg/kg HP-ß-CD to pregnant rabbits caused a slight maternal and embryotoxicity but no teratogenicity.

In a 2-year carcinogenicity study in which rats were orally dosed with 0, 500, 2000, and 5000 mg/kg/day with HP-ß-CD, the only adverse effect noted was an increase in the weight of the pancreas.[263] Histological examination of the pancreatic tissue revealed a dose-related increase in hyperplastic and neoplastic changes in the acinar cells of the exocrine pancreas. In separate and shorter studies with mice and dogs, no adverse effects were observed for the pancreas. The neoplasia in the rat study is inconsistent with the mutagenicity assay results and with the lack of carcinogenicity of the parent CDs.

Rat pancreatic hyperplasia is probably due to the ability of high concentrations of HP-ß-CD to increase fecal elimination of bile salts indirectly, stimulating production of cholecystokinin (CCK). In rats, CCK functions as a mitogen, increasing cellular hyperplasia in the acinar cells. Sensitivity to this effect is species dependent[271]; rats are most sensitive and dogs show no effects.[264]

4. Potential for Increased Elimination of Endogenous Lipophiles: An Explanation for Rat Pancreatic Hyperplasia

Neoplastic and hyperplastic changes of acinar cells in rat pancreas similar to those induced by HP-ß-CD were observed from treatment with agents that increase circulating cholecystokinin (CCK). CCK is a 33 amino acid peptide hormone that stimulates pancreatic secretion. The pancreas secretes enzymes into the intestine to digest proteins, carbohydrates, and fats. CCK is released from the jejunum (the section of the small intestine between the duodenum and ileum) into circulation in response to a decrease in intestinal trypsin activity and luminal bile acids.

Trypsin activity can be artificially reduced by consumption of foods that contain trypsin inhibitors, and this stimulates CCK release. Raw soya flour contains trypsin inhibitors,[265] and feeding rats a diet contain raw soya flour[266] results in an increase in circulating CCK levels.[267] This produces hypertrophy and hyperplasia of the pancreas through enhanced stimulation of pancreatic secretions; if this stimulation continues chronically, pancreatic adenomas and carcinomas may develop.

Similarly, decreased bile acids in the intestinal lumina caused a disinhibition of the feedback mechanism controlling production of CCK, resulting in increased production of the hormone. Cholestyramine, an ion exchange resin, has been used to absorb bile salts in the intestinal lumen, decreasing their enterohepatic recirculation.[268,269] Chronic administration of cholestyramine increased circulating CCK levels in rat, resulting in pancreatic hypertrophy and hyperplasia similar to that observed for treatment of rats with 2000 or 5000 mg/kg HP-ß-CD in the 2-year study.

DeCaprio et al.[270] demonstrated that an HP-ß-CD solution (45% wt/vol) readily solubilized cholesterol (15.5 µmol/ml), cholesterol metabolites, and bile salts (typically > 50 µmol/ml). The ability of HP-ß-CD to solubilize bile salts and yet not be metabolized in the intestinal tract suggests that HP-ß-CD should be more effective at increasing fecal elimination of bile salts than would ß-CD, which was metabolized in the colon and which has not been shown to produce pancreatic hyperplasia. Elimination of bile salts results in increased levels of CCK, which have already been shown to produce pancreatic hyperplasia.

Support for this indirect action of HP-ß-CD on the pancreas was provided by Van Cauteren et al.[263] A CCK-antagonist prevented the pancreatic hyperplasia resulting from an increase in circulating CCK levels.[271] A 1-month treatment of rats with 5000 mg/kg HP-ß-CD with and without the CCK antagonist (10mg/kg) was compared to a control group receiving the same dose of the CCK antagonist. HP-ß-CD resulted in an increase in the weight of the pancreas, but the group ingesting HP-ß-CD with the CCK antagonist exhibited lower pancreatic weights comparable to those of the CCK antagonist control group. Bile salts were also encapsulated by HP-ß-CD in the intestinal tract of the rats. Both results suggest that the neoplastic changes observed in the 2-year rat carcinogenicity study with HP-ß-CD were a result of sequestration of the bile salts. The neoplastic effect of increased CCK levels has not been reported in humans, even though other CCK-enhancing agents such as cholestyramine have been used chronically in man.[267]

5. *Parenteral Administration: General Safety*

Introduction of the hydroxypropyl substituent was more successful in eliminating systemic toxicity of parent and methylated CDs.

a. Absorption, Distribution, Metabolism, and Excretion

Frijlink et al.[227] followed the pharmacokinetics of intravenous administration of 100 and 200 mg/kg doses of HP3-ß-CD to rats. Greater than 96% of the dose was recovered unmetabolized in the urine 24 hours postadministration. Clearance was similar to that of inulin, a polysaccharide known to rapidly distribute in extracellular fluid with excretion at the glomerular filtration rate.

Monbaliu et al.[259] administered ^{14}C-HP-ß-CD intravenously to rats and dogs from 50, 100, 200, and 400 mg/kg doses from a 20% solution. Ninety percent of the radiolabelled HP-ß-CD from a single dose of 200 mg/kg was excreted unmetabolized in the urine, with minimal excretion in the feces and expired air. Half life in the plasma and total plasma clearance was 0.4 hr and 512 ml/kg/hr for rats and 0.8 hr and 188 ml/kg/hr for dogs, which corresponds to rapid urinary clearance comparable to the glomerular filtration rate. Plasma concentration increased linearly with increasing doses of 50, 100, and 400 mg/kg, but the pharmacokinetics were not affected by repeat administration of daily doses for 90 days.

Tissue distribution of ^{14}C-HP-ß-CD was limited in both rats and dogs, and the distribution profile was that expected for a compound that confines itself to the circulatory system with urinary elimination. A single dose of ^{14}C-HP-ß-CD was highest in the kidney and lungs of the rats. In a 30-day treatment of dogs at 825 mg/kg, concentration of ^{14}C-HP-ß-CD was highest in the kidney and liver.

Szathmary et al.[260] found that humans handle HP-ß-CD in a similar manner. IV doses of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 grams of HP-ß-CD were administered to human volunteers at an infusion rate of 1 gm/min. The escalating doses were administered to each volunteer following a 1-week wash-out period. Plasma half-life of the HP-ß-CD was 1.4–1.8 hrs. Renal clearance was 110-130 ml/min, was dose independent, and is nearly equivalent to human glomerular filtration rate. Approximately 82% of the dose was eliminated in the urine 24 hours after dosing.

Because subchronic safety studies on rats indicate that the kidney may be temporarily effected by treatment with HP-ß-CD, extensive evaluation of kidney function was conducted during the human trial described above. Seiler et al.[272] evaluated the urinary levels of alanine aminopeptidase (AAP), γ-glutamyl transpeptidase (γ-GT), N-acetyl-ß-glucoamidase (ß-NAG), creatinine, and protein—all markers of

kidney function. No changes were observed in kidney function for humans treated with escalating doses (0.5–3 gms) of HP-ß-CD.

b. Renal Effects

Anderson et al.[273] observed no effects from a single IV injection of 2 gm/kg to adult rats. Pitha et al.[274,231] observed no mortalities in mice injected intraperitoneally with 10 gm/kg HP5-α-CD, HP4-ß-CD, HP6-ß-CD, or HP5-γ-CD. When HP-ß-CD was administered intravenously,[275] no mortalities were observed, and the only effect of the treatment was a slight hematuria for single IV doses of 10 gm/kg.

Anderson et al.[273] treated Sprague-Dawley rats intravenously for 14 days with daily doses of 100 mg/kg or bi-daily doses of 200 mg/kg. Asaline and a carbohydrate control group were studied, in which molar equivalent doses of mannitol were administered. No effects were observed in the animals' health or on gross necropsy of the organs. Brewster et al.[275] followed these acute evaluations with 14- and 90-day intravenous safety studies on HP7-ß-CD in rats and monkeys. CD was dosed either as a 20% or 50% (w/v) solution, to administer a dose of 200 mg/kg every second day. A 23% solution of HP7-ß-CD is isotonic. No significant effects were observed in the 14-day study on body weight, food consumption, hematology, blood chemistry, or urinalysis. The only effects reported for the 90-day rat study were a slight increase in serum creatinine, a small decrease in MCV in male rats, and a slight increase in WBC and lymphocytes in female rats. Treatment of monkeys with 200 mg/kg every second day for 90 days was similarly uneventful.

Coussement et al.[261] reported on the 90-day treatment of rats and dogs with daily intravenous doses of 50, 100, and 400 mg/kg. The animals were evaluated for changes in body weight, hematology, blood biochemistry, urinalysis, gross pathology, organ weight, and histopathology. No adverse effects were observed in the rats at the 50mg/kg dose. At the 100 mg/kg dose, the rats exhibited swollen epithelial cells in the urinary bladder, swollen and granular kidney tubule cells, and a slight increase in the Kupffer cells in the liver. These effects were observed for the 400 mg/kg dose, plus there was a small decrease in body weight, a decrease in food consumption, and an increase in water consumption.

Hematological changes in the high-dose group included decreases in hematocrits, hemoglobin, and red blood cells and increases in creatinine, total bilirubin, aspartate, and alanine amino transferase serum levels. Urinalysis showed an increase in leukocytes, cylindrical epithelial cells, occult blood, and granular casts. Loss of blood cells and increase in bilirubin suggest that blood cells were damaged by the treatment. This seemed to be verified by an increase in the weight and red-pulp hyperplasia of the spleen, in the Kupffer cells, and in the reticuloendothelial system

(RES) aggregates of the liver. One of the functions of the spleen is to remove old and damaged blood cells from circulation, and liver Kupffer cells and the liver RES are primarily responsible for particulate and microbial clearance from the blood.

Increased weights were observed for the adrenals and kidneys, and histological changes occurred with an increase in lung foam cells. All of these changes were reversible upon cessation of treatment, except that serum enzyme levels were still slightly elevated and there was still a slightly higher number of pulmonary foam cells and swollen epithelial cells in the bladder.

The swollen epithelial cells and histologic changes observed in the kidneys were similar to the first changes produced from the intravenous administration of α- and ß-CD, but the further changes exerted by the parent CDs did not occur. The effects of HP-ß-CD were reversible and similar to those observed as an adaptive response to excretion of high concentrations of other osmotic agents such as glucose, mannitol, and dextran.[220-223] The difference in the intravenous effect between the HP-ß-CD and the parent CDs suggest that the mechanism of toxicity of the parent CD must involve factors beyond the occurrence of an increase of the vacuoles.

Increase in adrenal weight during intravenous administration of HP-ß-CD to animals caused concern that extensive elimination of HP-ß-CD in the urine may enhance renal elimination of adrenal-cortical hormones. Therefore, testosterone, cortisol, and aldosterone plasma concentrations were measured before and 30, 60, and 120 minutes postadministration. No changes were observed in plasma hormonal concentrations, and there was no change in the urinary excretion of cortisol.

No adverse effects were observed in dogs at 50 or 100 mg/kg. The 400 mg/kg doses produced increases in serum alanine (ALT) and aspartate aminotransferase (AST) activities and in serum bilirubin, but both of these affects normalized when the dogs were allowed a 1-month recovery period. (Alanine transaminase ALT = glutamic-pyruvic transaminase, formerly referred to as GPT or SGPT; aspartate transaminase AST = glutamic-oxaloacetic transaminase, formerly referred to as GOT or SGOT.) The only histological observations were a slight increase in pulmonary foam cells, swollen epithelial cells of the urinary bladder, and renal pelvis epithelium. The first two changes were completely reversible on cessation of treatment for 1 month, but the renal changes were only partially reversed.

Human studies have been conducted for intravenous administration of 0.5–3 gm of HP-ß-CD at an infusion rate of 100 mg/min. Extensive attention was given to evaluating kidney functions. In addition to general clinical evaluations, the urinary markers for renal safety—γ-glutamyl transpeptidase (γ-GT), N-acetyl-ß-D-glucosaminidase (ß-NAG), alanine amino peptidase (AAP), total protein, albumin, and creatinine clearance—were measured at 24, 48, and 72 hours postadministration. There were no effects on any of the markers of renal function for administration of up to 3 grams of HP-ß-CD.

c. Cytotoxicity: Hemolysis and Tissue Irritation

Yoshida et al.[248] evaluated the effect of neutral modified dimethyl and hydroxyalkyl CDs on human erythrocytes, and Figure 22 (see page 64) showed that the damaging effects are in the order of DM-ß-CD ≫ ß-CD > α-CD > HP-ß-CD > γ-CD.

Irie et al.[208] reported equilibrium lipid solubilities (refer to Table 12 on page 52) in various CD solutions and demonstrated that although the solubility of cholesterol in (2HP5)-ß-CD was greater than in ß-CD, HP-ß-CD does not cause as much damage to RBC. Hydroxypropyl CD solutions exhibit surface tensions[148] (~ 69 mN m^{-1}) comparable to water (~ 69 mN m^{-1}), but surface activity does increase, with an increase in degree of substitution, as evidenced by a decrease in the surface tension. For HP-ß-CD preparations with a DS of 2, 3, 4.38, 7.82, and 8.47, the surface tensions were 69.5, 65, 62, 59, and 58.5 mN m^{-1}, respectively.

Changing the degree of substitution for the hydroxypropyl derivative, however, did not appear to affect membrane destabilization. These results have also been observed for evaluating the isomeric hydroxyethyl and hydroxypropyl derivatives.[141] Introduction of the more hydrophilic dihydroxypropyl substituent did exhibit a decrease in hemolytic behavior, with an increase in degree of substitution. Figure 23 shows that the concentration of the CDs to effect 50% hemolysis steadily increased, with an increasing degree of substitution for DHP-ß-CD but not for HE or HP-

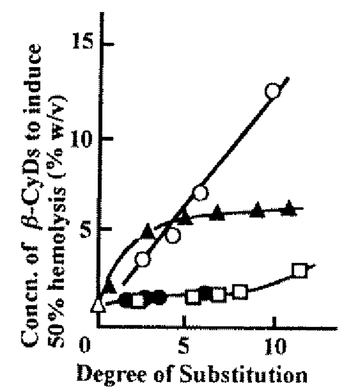


FIGURE 23. Relationship between hemolytic activity and degree of substitution[141] of hydroxyalkylated ß-CDs; Δ ß-CD; ● (3HP)-ß-CD; O (2, 3-DHP)-ß-CD; ▲ 2HE-ß-CD; □ 2HP-ß-CD. (Reprinted with permission of the *Chemical and Pharmaceutical Bulletin*, The Pharmaceutical Society of Japan)

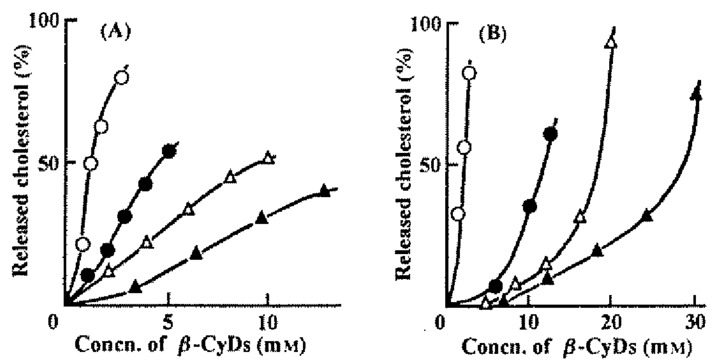


FIGURE 24. Release profiles of cholesterol (A) and protein (B) from human erythrocytes[141] treated with ß-CD or (2,3-DHP)-ß-CD with different degrees of substitution; O ß-CD; ● (2,3-HP)-ß-CD (MDS = 2.6); Δ (2,3-DHP)-ß-CD (MDS = 5.9); ▲ (2,3-DHP)-ß-CD (MDS = 9.3). (Reprinted with permission of the *Chemical and Pharmaceutical Bulletin*, The Pharmaceutical Society of Japan)

CDs. The effect of degree of substitution for DHP-ß-CD correlated with the release of cholesterol, as shown in Figure 24.

The ability of CDs to damage cellular membranes is not limited to their effect on erythrocyte membranes. Leroy-Lechat et al.[233] showed that cytotoxicity is not specific to cell type. The order of membrane-damaging activity of parent CDs with P388 murine leukæmic cells was the same as that observed for human erythrocytes. Bar and Ulitzur[234] reported similar results with the bacteria *E. coli*, with a toxicity order of DM-ß-CD ≫ ß-CD > HP-ß-CD > α-CD > γ-CD.

The membrane-damaging effect of the CDs can be observed *in vivo* upon IM injection. Damage to the *M. vastus lateralis* was evaluated by Yoshida et al.[141] according to the Shintani method, and the order of damage was DM-ß-CD ≫ α-CD > ß-CD > (2HP)4-ß-CD ~ (3HP)5-ß-CD > DHP5-ß-CD. Again, the more hydrophilic the CD, the less the muscular irritation.

No irritation was observed for intravenous, subcutaneous, intramuscular, or intraperitoneal injections of HP-ß-CD to rabbits, nor was there ocular irritation from the HP-ß-CD solution.[261] Brewster and Bodor[262] observed no irritation or abnormal histology in rat muscle injected with 5%, 10%, 20%, or 40% solution of HP-ß-CD.

D. Sulfobutylether-ß-CD and Anionic CD Derivatives

1. *Sulfate CDs: Safety Issues*

The observation that sulfated CDs exhibit pharmacological activity may have prompted Pitha's statement[152] that, to be safe excipients, CDs need to be polar but electrically neutral. Bernstein et al.[276] and Lewis and Bernstein[277,278] described the pharmacological activities of sulfated CDs in immunological cascades. Tetradecasulfate of ß-CD (S14-ß-CD) resembles heparin in its anticoagulant activity (Fig. 25), showing elongated blood clotting times with small changes in concentration.

Recent studies examined the use of sulfated CDs as antiviral agents,[279-285] angiogenesis inhibitors,[286-292] and inhibitors of smooth muscle cell proliferation.[293,294] Subsequent studies have shown, however, that not all ionic CDs exhibit the pharmacological activity demonstrated by sulfated CDs.

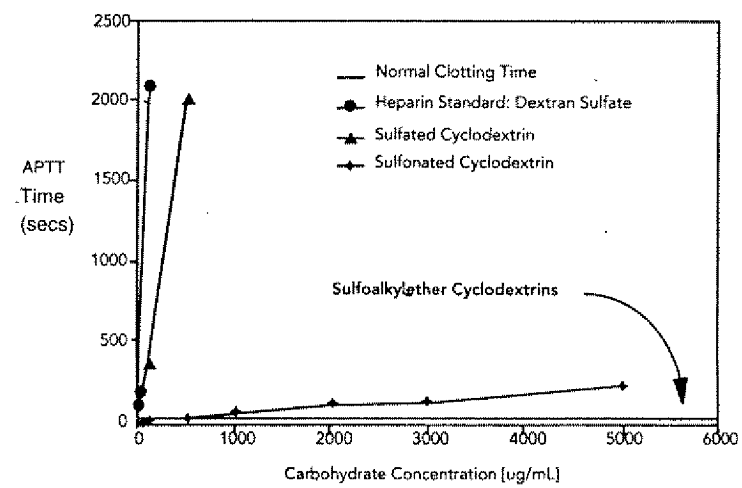


FIGURE 25. Effect of anionic CDs[337] on activated partial thromboplastin clotting time—dextran sulfate functions as a heparin standard.

2. Sulfobutylether-ß-CD

a. Pharmacologically Inactive

Unlike the sulfated CDs, sulfonate derivatives do not effectively participate in lengthening blood clotting times. The anticoagulant activity of directly sulfonated CDs 6-SA1-ß-CD or 6-SA7-ß-CD is dramatically reduced compared to tetradecasulfated ß-CD (Figure 25), and the sulfoalkyl derivatives cause no change in clotting times. The pharmacological activities observed for the sulfated CDs are not evidenced by the sulfonated derivatives.

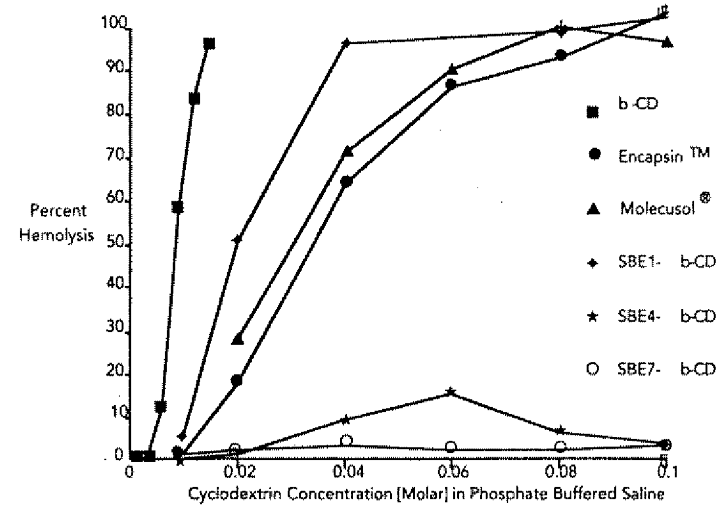
b. Parenteral Administration: General Safety

Renal Effects. Rajewski et al.[172] administered directly sulfonated, sulfopropyl, and sulfobutylether derivatives of ß-CD to mice IP and determined that > 10 gms/kg could be administered without toxic effect. Histopathology of the kidneys demonstrated no adverse effects. Absorption from the intraperitoneal cavity was verified by urinary recovery of greater than 80% of the dose less than 6 hours postadministration.

Cytotoxicity: Hemolysis and Tissue Irritation. Anionic CDs have been evaluated for their effects on erythrocytes. Macarak et al.[295] showed that tetradecasulfated CD (S14-ß-CD) exhibited no hemolytic effects at concentrations as high as 300 mg/mL. These results were confirmed by Shiotani et al.,[296] who showed that the order of lysis of rabbit erythrocytes was ß-CD > HP-ß-CD > SBE4-ß-CD > S14-ß-CD.

Jodál et al.[247] demonstrated that increasing the degree of substitution for anionic carboxymethyl-ß-CD decreased hemolytic behavior. Introduction of a single succinyl ester at a 3-hydroxyl in 2,6-DM14-ß-CD produced a mono-anion that exhibited significantly reduced hemolytic behavior compared to that exhibited by methylated CD.

Rajewski et al.[172] showed that increasing the molar degree of substitution of SBE-CDs reduced the effects of these anionic CDs on membrane destabilization. Figure 26 shows the hemolytic behavior of ß-CD, SBE1, SBE4, and SBE7, as well as HP4 and HP8 on a short incubation with human erythrocytes. Changing the degree of substitution for the hydroxypropyl derivative did not appear to affect membrane destabilization, but an increase in degree of substitution on anionic SBE derivatives substantially decreased lysis of the erythrocytes. The order of hemolysis is ß-CD > SBE1 ≤ Encapsin (HP4) = Molecusol (HP8) > SBE4 > SBE7.



pH 7.4, 37°C, 5 min Incubation

FIGURE 26. Hemolytic effects of CD derivatives[337] on human erythrocytes in isotonic phosphate buffer (pH 7.4) at 37° C for 5 mins; ■ ß-CD; ◆ SBE1-ß-CD; ● HP2-ß-CD; Δ HP4-ß-CD; ★ SBE4-ß-CD; O SBE7-ß-CD.

The shape of these hemolysis curves differ from those reported by Shiotani et al.,[296] but the two studies were conducted with different cell types (rabbit versus human erythrocytes) and with different incubation times. These experimental factors affect the shape of the hemolysis curves but not the order of hemolytic behavior.

Muscular damage has also been evaluated by measuring the levels of creatinine kinase released in serum from intramuscular injections of saline and SBE-ß-CD solution versus a cosolvent mixture (polyethylene glygol/ethanol) typically used to formulate water-insoluble drugs.[176] The saline and SBE-ß-CD solutions produced comparable levels of creatinine kinase, both of which were significantly lower than that observed for (often irritating) injections of the polyethylene glygol/ethanol mixture.

The decreased hemolysis and tissue damage produced by anionic CDs may be explained in one of two ways: First, hemolysis studies with these derivatives were conducted at higher ionic strength, and hypertonic conditions are known to protect the erythrocyte; or second, anionic derivatives differ in their ability to interact with

the cell membrane for removal of cholesterol, and this difference may, in turn, cause a difference in the ability to extract lipid from the membrane.

The mono-, tetra-, and hepta-substituted anionic SBE-CDs solubilized cholesterol according to the degree of substitution (refer to Fig. 20 on page 44). The phase solubility diagrams show the same order for solubilization as was observed for the order of CD hemolysis behavior. The decreasing ability of higher substituted SBE derivatives to solubilize cholesterol was explained by their inability to form higher-order complexes with cholesterol.

Although no oral safety studies have been reported for SBE7-ß-CD, this derivative is expected to exhibit low absorption and high excretion in the feces. Complexing bile salts by anionic SBE7-ß-CD is expected to be less favorable than by neutral CDs because the anionic charge at the end of the bile salt may repel anionic CD. This repulsion may limit 1:1 complexation, and the inability of SBE7-ß-CD to form 1:2 complexes with steroids may lessen the excretion of bile salts from the GI tract.

E. Summary

CDs are not absorbed upon oral admininstration and consequently exhibit a good oral safety profile. The main adverse effect observed with oral use occurs at very high doses and results from a secondary effect caused by removal of bile salts from enterohepatic recirculation. This effect is not observed at doses utilized in pharmaceutical formulations. Parent CDs α- and ß-CD are not suitable for systemic formulations because of renal toxicity, but nephrotic damage is not observed with γ-CD, HP-ß-CD, or SBE-ß-CD. All of the CDs should be suitable for use in transdermal and transmucosal delivery. Because of the membrane-damaging effects of DM-ß-CD, it will probably see use as only as a penetration enhancer.

VII. REGULATORY STATUS: CYCLODEXTRINS ARE NEW EXCIPIENTS

The previous sections clearly demonstrated the ability to produce safe, high-quality CDs for use in pharmaceutical formulations. The introduction of 10 commercial CD-based formulations in Japan and Europe confirm that CDs can pass regulatory reviews. Although a 30-year research database is available on CDs, these materials are still considered new excipients. The current and future regulatory situation facing both new and old excipients requires an examination of the functions of global regulatory agencies, pharmacopeias, and various other pharmaceutical organizations.

One organization leading the discussion on the regulatory situation facing excipients is the International Pharmaceutical Excipients Council (IPEC),* which was established in 1992 with counterpart organizations in the United States, Europe, and Japan. IPEC's objective is to harmonize pharmacopeial standards, GMP guidelines to ensure quality, and safety evaluation guidelines for developing new excipients.

IPEC is working on these objectives in coordination with representatives from the major pharmacopeias—the United States Pharmacopeia (USP), the European Pharmacopeia (EP), and the Japanese Pharmacopeia (JP)—and the respective regulatory agencies—the United States Food and Drug Administration (FDA), the European Community Committee on Proprietary Medicinal Products, the Japanese Pharmaceutical Affairs Bureau of the Japanese Ministry of Health and Welfare (MHW), and the National Institute of Hygienic Sciences (NIHS). Clearly, all segments of the pharmaceutical industry recognize the importance of new excipients like the CDs, and issues of quality and safety are crucial to their regulatory acceptance.

A. Regulatory Concerns for Excipients: Quality and Safety

1. *CD Quality Status*

The quality of inactive ingredients is of growing concern to the pharmaceutical industry. The ability of manufacturers to produce a consistent, defined CD preparation is essential. The material must be defined in terms of chemical identity, production quality, and safety. Chemical definition and production quality are established in the chemistry, manufacturing, and control (CMC) section of each drug master file. The manufacturer provides information on the identity and characterization of the CD and on the impurity profile, and provides data to indicate that production is controlled for batch-to-batch consistency. Although CMC information is confidential for each CD supplier, the scientific literature has clearly established the ability to define and control CD preparations for generation of a quality material.

---

* a) IPEC-Americas, Bldg. 3, 1361 Alps Road, Wayne, NJ 07470, U.S.A., (201) 628-3231.
b) JPEC-Americas, Japan Pharmaceutical Excipients Council, Kanda Chuo Building 701, 5-7 Nishikanda, 2-chome, Chiyoda-ku, Tokyo, 101 Japan, Fax +81 353 75 9517 or +81 352 75 5418.
c) IPEC-Europe, Aqualon France, 3 rue E. et A. Peugeot, 92508 Rueil Malmaison Cedex, France, +47 10 24 00.

2. CD Safety Status

Although no regulations exist to guide the safety evaluation of new excipients, the safety section of this review has clearly outlined the extensive database of safety studies on CDs, and the results show them to be safe for the indicated uses. In many cases, the safety database on a CD is as extensive as the global guidelines for toxicity testing of pharmaceutical (active) ingredients.

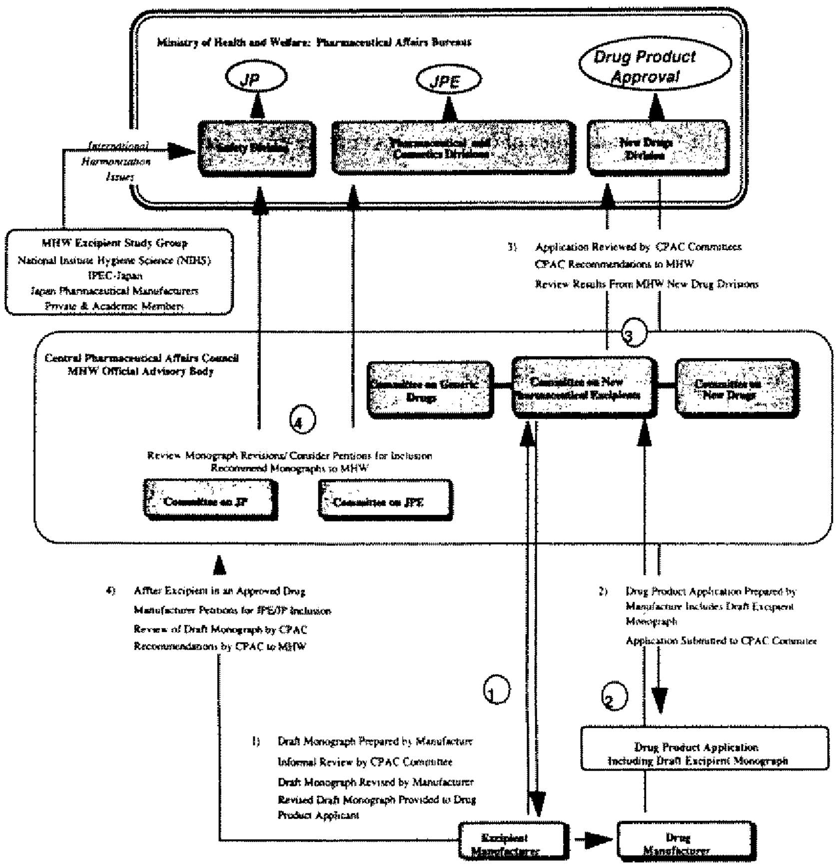


FIGURE 27. Schematic of regulatory review of excipients in Japan.

B. Current Regulatory Process for New Excipients

Hesitation about using CD formulations in clinical studies is mainly due to uncertain regulatory acceptance of formulations containings "nonstandard" inactive ingredients. A common perception exists that an approval process is in place for evaluating new excipients. In fact, there is no mechanism for submission and review of data on a new excipient that would lead to its approval.[298] Globally, regulatory authorities are charged with evaluating and approving final commercial drug formulations, but they are *not* charged with approval of new excipients.[299]

1. Regulatory Situation in Japan

The specific regulatory situation facing new excipients is unique to each global area, but all systems share common features. The flow scheme in Figure 27 describes the regulation of excipients in Japan.[300] The dossier on a new excipient is evaluated when an application for a drug dosage form containing the excipient is submitted. The data is evaluated in terms of both the excipient and the active ingredient, but only the drug product is approved.

In Japan, the process provides for inclusion of the monograph on the new excipient in the Japanese Pharmaceutical Excipients (JPE) compendium, formerly the Japanese Standards of Pharmaceutical Ingredients (JSPI). The JPE describes a set of voluntary standards and specifications that establish the quality of the inactive ingredient. Although the Japanese system has established regulatory authority over the quality of inactives used in formulations, meeting the specifications of quality does not ensure acceptance of the inactive ingredient in a formulation.

After the excipient has been used extensively in multiple marketed products, the regulatory process calls for a review of the data resulting in possible inclusion of the monograph in the Japanese Pharmacopoeia (JP). The JP defines mandatory standards for substances used in a pharmaceutical product. Inclusion in the JP establishes "precedent" status for the excipient, and this notation permits its use in new drug products under defined conditions without the need to submit extensive supporting data.

2. Regulatory Situation in the United States and Europe

The situation (Fig. 28) in the United States and Europe is somewhat similar to that in Japan. The regulatory agency (in the United States, the FDA) reviews a new ex-

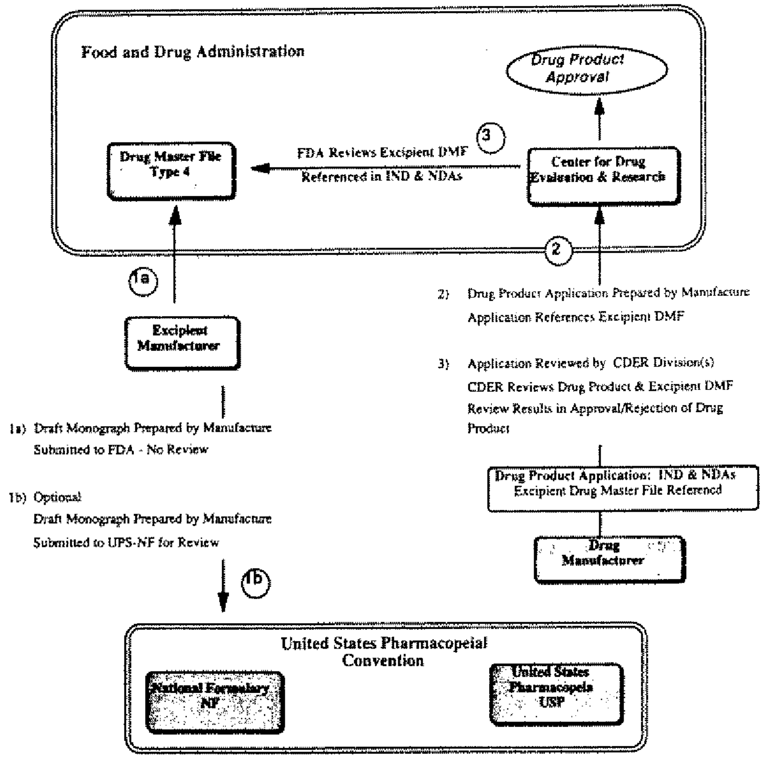


FIGURE 28. Schematic of regulatory review of excipients in United States.

cipient only as it applies to the review of a drug formulation. As in Japan, only the final drug product is approved. By this method, the excipient data is reviewed with each drug application. The dossier on a new excipient is filed by the excipient manufacturer as a drug master file (DMF)-Type 4.[301] This data is then referenced when an investigational new drug application (IND) or new drug application (NDA) is filed for a drug dosage form using the excipient.

There is a perception that a process exists for review of a new excipient and its inclusion in a list commonly referred to as "generally recognized as safe," or GRAS. The GRAS list[302] actually applies only to food additives that are reviewed by the FDA and determined to be generally recognized as safe for the purpose and use conditions described in the statute. The use of these excipients is frequently, but not always, transferable to oral pharmaceutical formulations. A petition can be filed for GRAS status to evaluate a new material as a food additive. Once the material is approved for use in foods, it may be considered suitable for use in an oral formulation if the dose fits within the quantities consumed as a food additive.

Currently there is no method for approval of new pharmaceutical excipients by the FDA except as food additives. No process exists for review and approval of new excipients to be used by non-oral routes of administration, yet non-oral products account for 30% of pharmaceutical sales in 1993, and these product areas are growing. Thus there is a major need for excipients for all routes of administration, not just the oral route.

In contrast to the Japanese system, which links the regulatory review of excipient dossiers to inclusion in the pharmacopeia, there is no regulatory link between the FDA review of new excipients and their inclusion in the United States Pharmacopeia-National Formulary (USP-NF). The USP-NF is published by the United States Pharmacopeial Convention, a private organization that defines quality standards for drug substances and dosage forms in the USP and other pharmaceutical ingredients in the NF.

The NF does have a process for accepting draft monographs on new excipients, which involves publication in the *Pharmacopeial Forum* for solicitation of public comment, followed by review and consideration for inclusion in the NF. The USP-NF carries no regulatory authority, but the compendia are officially recognized in various federal and state statutes as the source of standards for determining the strength, quality, and purity of medicinals. Acceptance of a new excipient monograph in the USP-NF provides a certain level of assurance in the chemical and manufacturing quality of the material but does not necessarily address safety and regulatory acceptance of the new excipient.

C. Current Regulatory Situation of CDs

In Japan, parent CDs are classified as natural starches and have received approval by the Ministries of Health for use in foods. Relative to pharmaceutical applications, monographs for α- and β-CD have been included in the Japanese Pharmaceutical Excipients (JPE)[86] compendium. Even though 9 pharmaceutical products with CD formulations have been marketed in Japan, use of CDs has not been extensive enough in approved formulations to receive precedent status.

In the United States, drug master files have been or will be submitted for each of the 6 commercial CDs discussed in this review. These DMFs are available for referencing in IND and NDA applications through agreements with the individual manufacturers.

CD manufacturers have defined the quality of ß-CD through publication of a monograph[303] in the XVIII issue of the NF. The USP-NF is reviewing proposals to include monographs on additional derivatives.

D. Future Regulatory Process for New Excipients: US Drug Master Files

Under the current situation in the United States, regulatory agencies are repeatedly evaluating excipient dossiers, causing additional work for reviewers and uncertainty for acceptability of the excipient in new formulations. The FDA is considering modifications to the current drug master file (DMF) system that may provide extensive review of a new excipient, with potential assignment of an "authorization" status. This process would be similar to the precedent status assigned to certain excipients in Japan.

Although the proposals were initially suggested for active ingredients, IPEC has recommended and the FDA is considering a similar classification system for DMFs on inactive ingredients. The proposals under review would classify DMFs into two types, Type A for substances referenced in NDAs and ANDAs, and Type B for substances referenced in INDs and unapproved marketing applications.[304] Extension of this system would provide a thorough review of the excipient's DMF-Type 4A dossier by the FDA. With a satisfactory review, the agency would grant regulatory authorization for suitability of an excipient in a given type of dosage form, by given route(s) of administration, and at defined dosage level(s). Excipients granted Type A status would have assurance that their use would not impede review of a new product formulated under the boundaries defined by the authorization. If implemented, this new system will provide a method by which the CDs could receive approval for use in a commercial formulations.

VIII. CONCLUSIONS

Solubility and stability issues continue to be major formulation obstacles hindering the development of therapeutic agents. CDs are enabling excipients; their ability to complex drugs enables the creation of formulations for water-insoluble drugs typically difficult to solubilize, stabilize, and deliver with more traditional additives. Complexing drugs by CDs can often minimize adverse side-effects of the active ingredient.

A CD-based formulation faces the same regulatory hurdles as other formulations. The introduction of 10 commercial CD-based formulations in Japan and Europe confirms that CD formulations can pass regulatory reviews.

The parent CDs (α, ß-, and γ-CD) and 3 modified derivatives of ß-CD, methyl (M), hydroxypropyl (HP), and sulfobutylether (SBE), are commercially available in a quality suitable for pharmaceutical formulations. Safety evaluations demonstrate that each CD may be suitable for use in oral formulations. Formulators now have CDs (γ-CD, HP-ß-CD, and SBE7-ß-CD) to use in parenteral and oral formulations. All of the CDs discussed should have application in topical/transdermal and transmucosal formulations, and DM-ß-CD may see use as a penetration enhancer. We should see a growth in the number of commercial products using CD-based formulations.

ACKNOWLEDGMENTS

The author would like to thank Dr. V. J. Stella for the opportunity to participate in the studies on the SBE-CDs. The author is grateful to Drs. H. Lambert and T. Thompson for providing suggestions and comments during preparation of this manuscript. Thanks also are due to Mr. P. Higuchi for encouraging this project and Ms. C. Tuttle for her assistance in preparing the manuscript.

AUTHOR'S NOTE

All animals used in the author's investigations have been cared for according to the Animal Rights Act and the NIH Guide for Care and Use of Laboratory Animals. The author has and will receive benefits from a commercial party directly and indirectly related to the subject matter of this paper.

REFERENCES

(1) Proceedings of the 1st International Symposium on Cyclodextrins, in *Advances in Inclusion Science,* Szejtli, J., Ed., Kluwer Academic Publishers, Dordrecht, 1982.

(2) Proceedings of the 3rd International Symposium on Clathrate Compounds and Molecular Inclusion Phenomena and the 2nd International Symposium on Cyclodextrins, in *Advances in Inclusion Science,* Atwood, J. L., Davies, J. E. D., and Osa, T., Eds., Kluwer Academic Publishers, Dordrecht, 1984.

(3) Inclusion Phenomena in Inorganic, Organic, and Organometallic Hosts: Proceedings of the 4th International Symposium on Inclusion Phenomena and the 3rd International Symposium on Cyclodextrins, in *Advances in Inclusion Science,* Atwood, J. L. and Davies, J. E. D., Eds., Kluwer Academic Publishers, Dordrecht, 1987.

(4) *Proceedings of the 4th International Symposium on Cyclodextrins,* Huber, O. and Szejtli, J., Eds., Kluwer Academic Publishers, Dordrecht, 1988.

(5) *Minutes of the 5th International Symposium on Cyclodextrins,* Duchêne, D., Ed., Editions de Santé, Paris, 1990.
(6) *Mins of the 6th International Symposium on Cyclodextrins,* Hedges, A. R., Ed., Editions de Santé, Paris, 1992.
(7) *Seventh International Cyclodextrin Symposium Proceedings,* Osa, T., Hashimoto, H., Hattori, K., Hirai, H., Komiyama, M., Nagai, T., Nakamura, N., Takahashi, K., Takahashi, T., Takayama, K., Toda, F., Ueda, H., Ueno, A., and Uekama, K., Eds., Business Center for Academic Societies, Tokyo, 1994.
(8) **Szejtli, J.,** *Cyclodextrins and their Inclusion Complexes,* Budapest: Akadémiai Kiadó, 1982.
(9) *Cyclodextrins and their Industrial Uses,* Duchêne, D., Ed., Editions de Santé, Paris, 1987.
(10) *New Trends in Cyclodextrins and Derivatives,* Duchêne, D., Ed., Editions de Santé, Paris, 1991.
(11) **Frömming, K. and Szejtli, J.,** Cyclodextrins in pharmacy, in *Topics in Inclusion Science,* Davis, J. E. D., Iwamoto, T., Lipkowski, L., and Saenger, W., Eds., Kluwer Academic Publishers, Dordrecht, 1994.
(12) **Uekama, K. and Otagiri, M.,** Cyclodextrins in drug carrier systems, *CRC Crit. Rev. Ther. Drug Carrier Syst.,* 3, 1, 1987.
(13) **Croft, A. P. and Bartsch, R. A.,** Synthesis of chemically modified cyclodextrins, *Tetrahedron,* 39, 1417, 1983.
(14) **Szejtli, J.,** Medicinal applications of cyclodextrins, *Med. Res. Rev.,* 14, 353, 1994.
(15) **Uekama, K., Hirayama, F., and Irie, T.,** Application of cyclodextrins in pharmaceutical preparations, *Drug Targeting Deliv.,* 3, 411, 1994.
(16) **Hirai, S.,** Formulation studies of cefotiam hexetil hydrochloride: Effect of α-cyclodextrin as dissolution enhancer, in *7th International Cyclodextrin Symposium Proceedings,* Osa, T., Hashimoto, H., Hattori, K., Hirai, H., Komiyama, M., Nagai, T., Nakamura, N., Takahashi, K., Takahashi, T., Takayama, K., Toda, F., Ueda, H., Ueno, A., and Uekama, K., Eds., Business Center for Academic Societies, Tokyo, 1994.
(17) **Hirai, S., Koyama, H., Makino, T., Kitamori, N., and Toguchi, H.,** Formulation studies of a new oral cephalosporin, cefotiam hexetil hydrochloride [SCE-2174 (2 HCl)], *Yakuzaigaku,* 48, 189, 1988.
(18) **Muranushi, N., Yoshida, M., Kinoshita, H., Hirose, F., Fukuda, T., Doteuchi, M., and Yamada, H.,** Benexate-CD: Effect of inclusion compound formation on the antiulcer activity of benexate, the effective ingredient of benexate-CD, *Nippon Yakurigaku Zasshi,* 91, 377, 1988.
(19) **Suzuki, Y., Tsukada, T., Nagafuji, N., Tomoda, Y., Hayashi, T., Tanaka, H., Fujimoto, A., Nakajima, C., Shima, K., Ogura, T., and Takagishi, Y.,** Bitterness-suppressive formulation of benexate hydrochloride betadex prepared by hot melt granulation, *Yakuzaigaku,* 53, 201, 1993.
(20) **Hirano, K. and Shinoda, M.,** Benexate hydrochloride Betadex: An accelerated release antiulcer and antigastritis drug utilizing ß-cyclodextrin complex, in *7th International Cyclodextrin Symposium Proceedings,* Osa, T., Hashimoto, H., Hattori, K., Hirai, H., Komiyama, M., Nagai, T., Nakamura, N., Takahashi, K., Takahashi, T., Takayama, K., Toda, F., Ueda, H., Ueno, A., and Uekama, K., Eds., Business Center for Academic Societies, Tokyo, 1994.

(21) **Bettini, R., Santi, P., Catellani, P. L., Massimo, G., Bellotti, A., Barthelemy, C., Guyot-Hermann, A. M., Trublin, F., and Colombo, P.,** Permeation of piroxicam and piroxicam-ß-cyclodextrin through artificial membranes, *Eur. J. Pharm. Biopharm.,* 38, 203, 1992.
(22) **Santucci, L., Fiorucci, S., Chiucchiu, S., Sicilia, A., Bufalino, L., and Morelli, A.,** Placebo-controlled comparison of piroxicam ß-cyclodextrin, piroxicam, and indomethacin on gastric potential difference and mucosal injury in humans, *Digest. Dis. Sci.,* 37, 1825, 1992.
(23) **Nervetti, A., Ambanelli, U., and Ugolotti, G.,** Assessment of gastric mucosal damage by a new inclusion complex of piroxicam with ß-cyclodextrin: A functional study by a scintigraphic method, *J. Drug Dev.,* Suppl., 4, 39, 1991.
(24) **Patoia, L., Clausi, G., Farroni, F., Alberti, P., Fugiani, P., and Bufalino, L.,** Comparison of fecal blood loss, upper gastrointestinal mucosal integrity, and symptoms after piroxicam beta-cyclodextrin, piroxicam and placebo administration, *Eur. J. Clin. Pharmacol.,* 36, 599, 1989.
(25) **Warrington, S., Debbas, N., Farthing, M., Horton, M., and Umile, A.,** Piroxicam-beta-cyclodextrin: Effects on gastrointestinal blood loss and gastric mucosal appearance in healthy men, *Int. J. Tissue React.,* 13, 243, 1991.
(26) **Inaba, K. and Mizutani, J.,** Prediction of stability for prostaglandin E1-α-cyclodextrin complex, *Yakuzaigaku,* 46, 58, 1986.
(27) **Wiese, M., Cordes, H. P., Chi, H., Seydel, J. K., Backensfeld, T., and Müller, B. W.,** Interaction of prostaglandin E1 with α-cyclodextrin in aqueous systems: Stability of the inclusion complex, *J. Pharm. Sci.,* 80, 153, 1991.
(28) **Higuchi, T. and Conners, K. A.,** Phase solubility techniques, *Adv. Anal. Chem. Instr.,* 4, 117, 1965.
(29) **Hirayama, F. and Uekama, K.,** Methods of investigating and preparing inclusion compounds, in *Cyclodextrins and Their Industrial Uses,* Duchêne, D., Ed., Editions de Santé, Paris, 1987.
(30) **Qi, H., Nishihata, T., and Rytting, J. H.,** Study of the interaction between ß-cyclodextrin and chlorhexidine, *Pharm. Res.,* 11, 1207, 1994.
(31) **Durán-Merás, I., Muñoz de la Peña, A., Salinas, F., and Rodriguez Cáceres, I.,** Spectrofluorimetric determination of nalidixic acid based on host-guest complexation with γ-cyclodextrin, *Analyst,* 119, 1215, 1994.
(32) **Ndou, T., Mukundan, S. J., and Warner, I. M.,** Complexation of ephedrine with ß-cyclodextrin: An NMR spectroscopy study, *J. Inclusion Phenom. Molec. Recognit. Chem.,* 15, 9, 1993.
(33) **Alston, D. R., Ashton, P. R., Lilley, T. H., Stoddart, J. F., Zarzycki, R., Slawin, A. M. Z., and Williams, D. J.,** Second-sphere coordination of carboplatin and rhodium complexes by cyclodextrins (cyclomalto-oligosaccharides), *Carbohydr. Res.,* 192, 259, 1989.
(34) **Djedaïni, F. and Perly, B.,** Nuclear magnetic resonance investigation of the stoichiometries in ß-cyclodextrin-steroid inclusion complexes, *J. Pharm. Sci.,* 80, 1157, 1991.
(35) **Takisawa, N., Shirahama, K., and Tanaka, I.,** Interactions of amphiphilic drugs with α-, ß, and γ-cyclodextrins, *Colloid Polym. Sci.,* 271, 499, 1993.
(36) **DePonti, R., Torricelli, C., Motta, A., and Crivellente, M.,** Use of a polarographic method, a UV method, and the phase-solubility technique to determine the stability con-

stant in aqueous solution of a ß-cyclodextrin complex with a new immunomodulating agent, *Eur. J. Pharm. Biopharm.*, 37, 106, 1991.

(37) **Valsami, G. N., Koupparis, M. A., and Macheras, P. E.,** Complexation studies of cyclodextrins with tricyclic antidepressants using ion-selective electrodes, *Pharm. Res.*, 9, 94, 1992.

(38) **Koenigbauer, M. J.,** Pharmaceutical applications of microcalorimetry, *Pharm. Res.*, 11, 777, 1994.

(39) **Tong, W. Q., Lach, J. L., Chin, T. F., and Guillory, J. K.,** Structural effects on the binding of amine drugs with the diphenylmethyl functionality to cyclodextrins, I. A microcalorimetric study, *Pharm. Res.*, 8, 951, 1991.

(40) **Suzuki, M., Ueda, S., and Kusai, A.,** Application of freezing point depression to drug interaction studies, I. Interaction between cyclodextrins and alcohols, *Chem. Pharm. Bull.*, 36, 720, 1988.

(41) **Suzuki, M., Ito, K., Fushimi, C., and Kondo, T.,** Application of freezing point depression to drug interaction studies, I. A study of 1:1 plus 1:2 complexes between barbiturate and α-cyclodextrin using the freezing point depression method, *Chem. Pharm. Bull.*, 41, 1444, 1993.

(42) **Agnus, B., Sebille, B., and Gosselet, M.,** Effects of ß-cyclodextrin in the mobile phase on the retention and indirect detection of non-electrolytes in reversed-phase liquid chromatography, II. Steroids, *J. Chromatogr.*, 552, 583, 1991.

(43) **Thuaud, N., Sebille, B., Deratani, A., and Lelievre, G.,** Determination by high-performance liquid chromatography of the binding properties of charged ß-cyclodextrin derivatives with drugs, *J. Chromatogr.*, 503, 453, 1990.

(44) **Csabai, K., Cserháti, T., and Szejtli, J.,** Interaction of some barbituric acid derivatives with hydroxypropyl-ß-cyclodextrin, *Int. J. Pharm.*, 91, 15, 1993.

(45) **Cserháti, T.,** Charge-transfer chromatographic study of the interaction of anticancer drugs with acetyl-ß-cyclodextrin, *Fresenius' J. Anal. Chem.*, 349, 751, 1994.

(46) **Cserháti, T. and Holló, J.,** Interaction of taxol and other anticancer drugs with hydroxypropyl-ß-cyclodextrin, *Int. J. Pharm.*, 108, 69, 1994.

(47) **Kraus, C., Mehnert, W., and Froemming, K. H.,** Interactions of ß-cyclodextrin with Solutol HS 15 and their influence on diazepam solubilization, *PZ Wiss.*, 4, 11, 1991.

(48) **Lin, S. Y., Kao, Y. H., and Yang, J. C.,** Grinding effect on some pharmaceutical properties of drugs by adding ß-cyclodextrin, *Drug Dev. Ind. Pharm.*, 14, 99, 1988.

(49) **Abdel-Rahman, S. I. and El-Sayed, A. M.,** Interaction of some benzothiadiazine diuretics with ß-cyclodextrin, *Acta Pharm. Hung.*, 60, 69, 1990.

(50) **Chun, I. K. and Yun, D. S.,** Inclusion complexation of hydrocortisone butyrate with cyclodextrins and dimethyl-ß-cyclodextrin in aqueous solution and in solid state, *Int. J. Pharm.*, 96, 91, 1993.

(51) **Bekers, O., Beijnen, J. H., Otagiri, M., Bult, A., and Underberg, W. J. M.,** Inclusion complexation of doxorubicin and daunorubicin with cyclodextrins, *J. Pharm. Biomed. Anal.*, 8, 671, 1990.

(52) **Husain, N., Ndou, T. T., Muñoz de la Peña, A., and Warner, I. M.,** Complexation of doxorubicin with ß- and γ-cyclodextrins, *Appl. Spectrosc.*, 46, 652, 1992.

(53) **Otero-Espinar, F. J., Anguiano-Igea, S., Garcia-Gonzalez, N., Vila-Jato, J. L., and Blanco-Mendez, J.,** Interaction of naproxen with ß-cyclodextrin in solution and in the solid state, *Int. J. Pharm.*, 79, 149, 1992.

(54) **Van der Houwen, O. A. G., Bekers, O., Bult, A., Beijnen, J. H., and Underberg, W. J. M.,** Computation of mitomycin C-γ-cyclodextrin complex stability constant, *Int. J. Pharm.*, 89, R5, 1993.

(55) **McDonald, C. and Faridah,** Solubilities of trimethoprim and sulfamethoxazole at various pH values and crystallization of trimethoprim from infusion fluids, *J. Parenter. Sci. Technol.*, 45, 147, 1991.

(56) **Hoshino, T., Uekama, K., and Pitha, J.,** Increase in temperature enhances solubility of drugs in aqueous solutions of hydroxypropyl cyclodextrins, *Int. J. Pharm.*, 98, 239, 1993.

(57) **Menard, F. A., Dedhiya, M. G., and Rhodes, C. T.,** Physicochemical aspects of the complexation of some drugs with cyclodextrins, *Drug Dev. Ind. Pharm.*, 16, 91, 1990.

(58) **Pitha, J. and Hoshino, T.,** Effects of ethanol on formation of inclusion complexes of hydroxypropyl cyclodextrins with testosterone or with methyl orange, *Int. J. Pharm.*, 80, 243, 1992.

(59) **Kralova, K. and Mitterhauszerova, L.,** Interactions of ß-cyclodextrin with steroid compounds in aqueous solutions, *Pharmazie*, 44, 623, 1989.

(60) **Reer, O. and Müller, B. W.,** The influence of cosolvents and surfactants on the complexation of dexamethasone with hydroxypropyl-ß-cyclodextrin investigated with a simplex lattice design, *Eur. J. Pharm. Biopharm.*, 39, 105, 1993.

(61) **Loftsson, T., Frilfriksdottir, H., Sigurlfardottir, A. M., and Ueda, H.,** The effect of water-soluble polymers on drug-cyclodextrin complexation, *Int. J. Pharm.*, 110, 169, 1994.

(62) **Villiers, A.,** Fermentation de fécule par le ferment butyrique, *Compt. Rend.*, 112, 536, 1891.

(63) **Schardinger, F.,** Thermophile bakterien aus verschiedenen nahrungsmittein und milch und die gebildeten produkte, wenn diese bakterien in nahrlosungen kultiviert werden, die kohlenhydrate enthalten, *Z. Untersuch. Nahr. Genussm.*, 6, 865, 1903.

(64) **French, D. and Rundle, R. E.,** The molecular weights of the Schardinger alpha and beta dextrins, *J. Amer. Chem. Soc.*, 64, 1651, 1942.

(65) **Freudenberger, K. and Cramer, F.,** Die konstitution der Schardinger dextrine alpha, beta, und gamma, *Z. Naturforsch.*, 3b, 464, 1948.

(66) **Saenger, W., Betzel, C., Hingerty, B., and Brown, G. M.,** Flip-flop hydrogen bridging bonds in beta-cyclodextrin—a general principle in polysaccharides?, *Angew. Chem.*, 95, 883, 1983.

(67) **Jozwiakowski, M. J. and Connors, K. A.,** Aqueous solubility behavior of three cyclodextrins, *Carbohydr. Res.*, 143, 51, 1985.

(68) **Coleman, A. W., Nicolis, I., Keller, N., and Dalbiez, J. P.,** Aggregation of cyclodextrins: An explanation of the abnormal solubility of ß-cyclodextrin, *J. Inclusion Phenom. Mol. Recognit. Chem.*, 13, 139, 1992.

(69) **Paduano, L., Sartorio, R., Vitagliano, V., and Costantino, L.,** Diffusion properties of cyclodextrins in aqueous solution at 25°C., *J. Solution Chem.*, 19, 31, 1990.

(70) **Breslow, R. and Halfon, S.**, Quantitative effects of antihydrophobic agents on binding constants and solubilities in water, *Proceedings of the National Academy of Science, U. S. A.*, 89, 6916, 1992.

(71) **Müller, B. W. and Albers E.**, Effect of hydrotropic substances on the complexation of sparingly soluble drugs with cyclodextrin derivatives and the influence of cyclodextrin complexation on the pharmacokinetics of the drugs, *J. Pharm. Sci.*, 80, 599, 1991.

(72) **Pedersen, M.**, Effect of hydrotropic substances on the complexation of clotrimazole with ß-cyclodextrin, *Drug Dev. Ind. Pharm.*, 19, 439, 1993.

(73) **Buvári, A. and Barcza, L.**, Complex formation of inorganic salts with ß-cyclodextrin, *J. Inclusion Phenom. Mol. Recognit. Chem.*, 7, 379, 1989.

(74) **Coleman, A. W. and Nicolis, I.**, Inorganic salt modulation of the aqueous solubility of ß-cyclodextrin, *Supramol. Chem.*, 2, 93, 1993.

(75) **Cox, G. S., Hauptman, P. J., and Turro, N. J.**, Dialkylaminobenzonitriles as fluorescence polarity probes for aqueous solutions of cyclodextrins, *Photochem. Photobiol.*, 39, 597, 1984.

(76) **Street, Jr., K. W.**, Cyclodextrin cavity polarity and chromatographic implications, *J. Liq. Chromatogr.*, 10, 655, 1987.

(77) **Mattsson, P., Mäkelä, M., and Korpela, T.**, Chromatographic determination of cyclodextrins on benzoylated polyacrylamide gels, *J. Chromatogr.*, 447, 398, 1988.

(78) **Koizumi, K., Utamura, T., Kuroyanagi, T., Hizukuri, S., and Abe, J.**, Analyses of branched cyclodextrins by high-performance liquid and thin-layer chromatography, *J. Chromatogr.*, 360, 397, 1986.

(79) **White, G., Katona, T., Zodda, J. P., and Eakins, M. N.**, Determination of the impurity profile of γ-cyclodextrin by high-performance liquid chromatography, *J. Chromatogr.*, 625, 157, 1992.

(80) **Vaitkus, R., Kacinskiené, A., Jasinskiené E., and Dienys, G.**, Mechanism of hydrolysis of maltose and ß-cyclodextrin in aqueous acetic acid solution, *Org. React. (Tartu)*, 26, 55, 1989.

(81) **Koizumi, K., Utamura, T., Kubota, Y., and Hizukuri, S.**, Two high-performance liquid chromatographic columns for analyses of malto-oligosaccharides, *J. Chromatogr.*, 409, 396, 1987.

(82) **McGinnis, G. D., Prince, S., and Lowrimore, J.**, The use of reverse-phase columns for separation of unsubstituted carbohydrates, *J. Carbohydr. Chem.*, 5, 83, 1986.

(83) **Mäkelä, M., Korpela, T., and Laakso, S.**, Colorimetric determination of ß-cyclodextrin: Two assay modifications based on molecular complexation of phenolphthalein, *J. Biochem. Biophys. Methods*, 14, 85, 1987.

(84) **Koizumi, K., Kubota, Y., Tanimoto, T., and Okada, Y.**, Determination of cyclic glucans by anion-exchange chromatography with pulsed amperometric detection, *J. Chromatogr.*, 303, 1988.

(85) United States Pharmacopeia 23 National Formulary 18, in *United States Pharmacopeia National Formulary*, 1994.

(86) *Japanese Pharmaceutical Excipients* 1993, Yakuji Nippo, Ltd., Tokyo, 1994.

(87) **Bender, H.**, Production, characterization, and application of cyclodextrins, *Adv. Biotechnol. Processes*, 6, 31, 1986.

(88) **Sicard, P. J. and Saniez, M. H.**, Biosynthesis of cycloglycosyltransferase and obtention of its enzymic reaction products, in Duchêne D., Ed., *Cyclodextrins and Their Industrial Uses*, Editions de Santé, Paris, 1987.

(89) **Schmid, G.**, Cyclodextrin glycosyltransferase production: Yield enhancement by overexpression of cloned genes, *Trends Biotechnol.*, 7, 244, 1989.

(90) **Binder, F., Huber, O., and Boeck, A.**, Cyclodextrin-glycosyltransferase from Klebsiella pneumoniæ M5a1: Cloning, nucleotide sequence and expression, *Gene*, 47, 269, 1986.

(91) **Sin, K. A., Nakamura, A., Kobayashi, K., Masaki, H., and Uozumi, T.**, Cloning and sequencing of a cyclodextrin glucanotransferase gene from Bacillus ohbensis and its expression in Escherichia coli, *Appl. Microbiol. Biotechnol.*, 35, 600, 1991.

(92) **Kitamoto, N., Kimura, T., Kito, Y., and Ohmiya, K.**, Cloning and sequencing of the gene encoding cyclodextrin glucanotransferase from Bacillus sp. KC201, *J. Ferment. Bioeng.*, 74, 345, 1992.

(93) **Hamamoto, T., Kaneko, T., and Horikoshi, K.**, Nucleotide sequence of the cyclomaltodextrin glucanotransferase (CGTase) gene from alkalophilic Bacillus sp. strain No. 38-2, *Agric. Biol. Chem.*, 51, 2019, 1987.

(94) **Nitschke, L., Heeger, K., Bender, H., and Schulz, G. E.**, Molecular cloning, nucleotide sequence and expression in Escherichia coli of the ß-cyclodextrin glycosyltransferase gene from Bacillus circulans strain no. 8, *Appl. Microbiol. Biotechnol.*, 33, 542, 1990.

(95) **Lawson, C. L., van Montfort, R., Strokopytov, B., Rozeboom, H. J., Kalk, K. H., Penninga, D., de Vries, G., Dijkhuizen, L., and Dijkstra, B. W.**, Protein engineering of cyclodextrin glycosyl transferase (CGTase) from Bacillus circulans strain 251, in *Mins 6th International Symposium Cyclodextrins*, Hedges, A. R., Ed., Editions de Santé, Paris, 1992.

(96) **Starnes, R. L., Amemiya, K., Jorgensen, P. L., and Jorgensen, S.**, Cloning and expression of the Thermoanaerobacter sp. cyclodextrin glycosyl transferase gene in Escherichia coli and Bacillus subtilis, in *Mins 6th International Symposium on Cyclodextrins*, Hedges, A. R., Ed., Editions de Santé, Paris, 1992.

(97) **Seydel, W., Binder, F., Schmid, G., Huber, O., and Boeck, A.**, Expression of the gene for cyclodextrin-glycosyltransferase from Klebsiella in E. coli, in *Proceedings of the 4th International Symposium on Cyclodextrins*, Huber, O. and Szejtli, J., Eds., Kluwer, Dordrecht, 1988.

(98) **Kimura, K., Takano, T., and Yamane, K.**, Molecular cloning of the ß-cyclodextrin synthetase gene from an alkalophilic Bacillus and its expression in Escherichia coli and Bacillus subtilis, *Appl. Microbiol. Biotechnol.*, 26, 149, 1987.

(99) **Saha, B. C. and Zeikus, J. G.**, Cyclodextrin degrading enzymes, *Starch/Staerke*, 44, 312, 1992.

(100) **Schmid, G., Huber, O. S., and Eberle, H. J.**, Selective complexing agents for the production of γ-cyclodextrin, in *Proceedings of the 4th International Symposium on Cyclodextrins*, Huber, O. and Szejtli, J., Eds., Kluwer, Dordrecht, 1988.

(101) **Okada, Y., Kubota, Y., Koizumi, K., Hizukuri, S., Ohfuji, T., and Ogata, K.**, Some properties and the inclusion behavior of branched cyclodextrins, *Chem. Pharm. Bull.*, 36, 2176, 1988.

(102) **Yamamoto, M., Aritomi, H., Irie, T., Hirayama, F., and Uekama, K.,** Biopharmaceutical evaluation of maltosyl ß-cyclodextrin as a parenteral drug carrier, *S.T.P. Pharm. Sci.*, 1, 397, 1991.

(103) **Yamamoto, M., Yoshida, A., Hirayama, F., and Uekama, K.,** Some physicochemical properties of branched ß-cyclodextrins and their inclusion characteristics, *Int. J. Pharm.*, 49, 163, 1989.

(104) **Rao, C. T., Fales, H. M., and Pitha, J.,** Pharmaceutical usefulness of hydroxypropyl cyclodextrins: *"e pluribus unum"* is an essential feature, *Pharm. Res.*, 7, 612, 1990.

(105) **Johnson, J. R., Shankland, N., and Sadler, I. H.,** Full assignment of the proton and carbon-13 NMR spectra of 2,3,6 -tri-O-methyl-ß-cyclodextrin, *Tetrahedron*, 41, 3147, 1985.

(106) **Spencer, C. M., Stoddart, J. F., and Zarzycki, R.,** Structural mapping of an unsymmetrical chemically modified cyclodextrin by high-field nuclear magnetic resonance spectroscopy, *J. Chem. Soc. Perkin Trans. 2*, 1323, 1987.

(107) **Tabushi, I., Nabeshima, T., Yamamura, K., and Fujita, H.,** 400 MHz two-dimensional NMR studies of cyclodextrin derivatives for proton and carbon-13 chemical shift determination, *Bull. Chem. Soc. Jpn.*, 60, 3705, 1987.

(108) **Yamamoto, Y., Onda, M., Takahashi, Y., Inoue, Y., and Chûjô, R.,** Two-dimensional NMR spectra of O-methylated cyclomalto-oligosaccharides: Assignment of proton and carbon-13 resonances and conformational analysis, *Carbohydr. Res.*, 170, 229, 1987.

(109) **Pitha, J., Szabo, L., and Fales, H. M.,** Reaction of cyclodextrins with propylene oxide or with glycidol: Analysis of product distribution, *Carbohydr. Res.*, 168, 191, 1987.

(110) **Rajewski, R. A.,** Development and evaluation of the usefulness and parenteral safety of modified cyclodextrins, Dissertation, University of Kansas. (Avail.: Univ. Microfilms Int. Order No. DA9110909 From: Diss. Abstr. Int. B 1991, 51.11.5332)

(111) **Reer, O. and Müller, B. W.,** Determination of the degree of substitution of chemically modified cyclodextrins by a microcalorimetric titration technique, *Int. J. Pharm.*, 104, 239, 1994.

(112) **Metzger, J. W., Jung, M., Schmalzing, D., Bayer, E., and Schurig, V.,** Analysis of cyclomalto-oligosaccharides (cyclodextrins) and derivatives thereof by ion-spray mass spectrometry, *Carbohydr. Res.*, 222, 23, 1991.

(113) **Pitha, J., Milecki, J., Fales, H., Pannell, L., and Uekama, K.,** Hydroxypropyl-ß-cyclodextrin: Preparation and characterization, effects on solubility of drugs, *Int. J. Pharm.*, 29, 73, 1986.

(114) **Tait, R. J., Skanchy, D. J., Thompson, D. P., Chetwyn, N. C., Dunshee, D. A., Rajewski, R. A., Stella, V. J., and Stobaugh, J. F.,** Characterization of sulfoalkyl ether derivatives of ß-cyclodextrin by capillary electrophoresis with indirect UV detection, *J. Pharm. Biomed. Anal.*, 10, 615, 1992.

(115) **Szejtli, J., Dosa, E., Liptak, A., Nansai, P., Jodal, E., Neszmelyi, A., and Janossy, L.,** Partially methylated ß-cyclodextrin derivatives, Hungarian Patent No. 22967, 1980.

(116) **Casu, B., Reggiani, M., and Sanderson, G. R.,** Methylated cycloamyloses (cyclodextrins) and their inclusion properties, *Carbohydr. Res.*, 76, 59, 1979.

(117) **Szejtli, J., Liptak, A., Jodal, I., Fugedi, P., Nanasi, P., and Neszmelyi, A.,** Synthesis and carbon-13 NMR spectroscopy of methylated beta-cyclodextrins, *Starch/Staerke*, 32, 165, 1980.

(118) **Koizumi, K., Kubota, Y., Utamura, T., and Horiyama, S.,** Analysis of heptakis(2,6-di-O-methyl)-ß-cyclodextrin by thin-layer, high-performance liquid and gas chromatography and mass spectrometry, *J. Chromatogr.*, 368, 329, 1986.

(119) **Kubota, Y., Tanimoto, T., Horiyama, S., and Koizumi, K.,** Isolation and characterization of heptakis(2,6-di-O- methyl)cyclomaltoheptaose and over-methylated homologs, *Carbohydr. Res.*, 159, 1989.

(120) **Wenz, G.,** Synthesis and characterization of some lipophilic per(2,6-di-O-alkyl) cyclomaltooligosaccharides, *Carbohydr. Res.*, 214, 257, 1991.

(121) **Voyksner, R. D., Williams, F. P., Smith, C. S., Koble, D. L., and Seltzman, H. H.,** Analysis of cyclodextrins using fast atom bombardment/mass spectrometry and tandem mass spectrometry, *Biomed. Environ. Mass Spectrom.*, 18, 1071, 1989.

(122) **Irie, T., Fukunaga, K., Pitha, J., Uekama, K., Fales, H. M., and Sokolowski, E. A.,** Alkylation of cyclomalto-oligosaccharides (cyclodextrins) with dialkyl sulfate-barium hydroxide: Heterogeneity of products and the marked effect of the size of macrocycle, *Carbohydr. Res.*, 192, 167, 1989.

(123) **Mischnick-Lübbecke, P. and Krebber, R.,** Characterization of cyclomalto-hexaose and -heptaose derivatives by the reductive-cleavage method, *Carbohydr. Res.*, 187, 197, 1989.

(124) **Luna, E. A., Bornancini, E. R., Thompson, D. O., Rajewski, R. A., and Stella, V. J.,** Fractionation and characterization of a mixture of sulfobutyl ether ß-cyclodextrins, *Pharm. Res.*, 12, 1995.

(125) **Tanimoto, T., Kubota, Y., Nakanishi, N., and Koizumi, K.,** Isolation and characterization of hexakis(2,6-di-O- methyl)cyclomaltohexaose and octakis(2,6-di-O-methyl)cyclomalto-octaose and their over-methylated homologs, *Chem. Pharm. Bull.*, 38, 318, 1990.

(126) **Mischnick, P.,** Determination of the patterns of substitution of hydroxyethyl- and hydroxypropylcyclomaltoheptaoses, *Carbohydr. Res.*, 233, 1989.

(127) **Pitha, J., Rao, C. T., Lindberg, B., and Seffers, P.,** Distribution of substituents in 2-hydroxypropyl ethers of cyclomaltoheptaose, *Carbohydr. Res.*, 200, 429, 1990.

(128) **Luna, E. A., VanderVelde, D., Tait, R. J., Thompson, D. O., Stella, V. J., and Rajewski, R. A.,** Isolation and magnetic resonance characterization of monsubstituted sulfobutyl ether ß-cyclodextrins, *Pharm. Res.*, 12, 1995.

(129) **Rao, C. T. and Pitha, J.,** Reactivities at the O-2, O-3, and O-6 positions of cycloamyloses in Hakomori methylation, *Carbohydr. Res.*, 209, 1991.

(130) **Boger, J., Corcoran, R. J., and Lehn, J. M.,** Cyclodextrin chemistry, selective modification of all primary hydroxyl groups of α- and ß-cyclodextrins, *Helv. Chim. Acta*, 61, 2190, 1978.

(131) **Irie, T., Fukunaga, K., Yoshida, A., Uekama, K., Fales, H. M., and Pitha, J.,** Amorphous water-soluble cyclodextrin derivatives: 2-hydroxyethyl, 3-hydroxypropyl, 2-hydroxyisobutyl, and carboxamidomethyl derivatives of ß-cyclodextrin, *Pharm. Res.*, 5, 713, 1988.

(132) Regioselective substitutions in cyclodextrins, Lindberg, B. and Pitha, J., inventors, Janssen Pharmaceutical N. V., assignee, WO 90/12035, Oct 18, 1990.

(133) **Rao, C. T., Pitha, J., Lindberg, B., and Lindberg, J.,** Distribution of substituents in O-(2-hydroxypropyl) derivatives of cyclomalto-oligosaccharides (cyclodextrins): Influence of increasing substitution, of the base used in the preparation, and of macrocyclic size, *Carbohydr. Res.*, 223, 99, 1992.

(134) **Luna, E., Tait, R. J., Thompson, D. O., Stella, V. J., and Stobaugh, J. F.,** Evaluation of the utility of capillary electrophoresis (CE) for the analysis of modified cyclodextrin mixtures, in *Proceedings of the 7th International Symposium on Cyclodextrins,* Osa, T., Hashimoto, H., Hattori, K., Hirai, H., Komiyama, M., Nagai, T., Nakamura, N., Takahashi, K., Takahashi, T., Takayama, K., Toda, F., Ueda, H., Ueno, A., and Uekama, K., Eds., Business Center for Academic Societies Japan, Tokyo, 198, 1994.

(135) **Liu, G., Goodall, D. M., and Loran, J. S.,** Quantification of trace levels of ß-cyclodextrin and substitution patterns in hydroxypropyl-ß-cyclodextrin using high-performance liquid chromatography with polarimetric detection, *Chirality,* 5, 220, 1993.

(136) **Ou, D., Ueda, H., Nagase, H., Endo, T., and Nagai, T.,** Some pharmaceutical properties of 2,3,6-partially methylated-ß-cyclodextrin and its solubilizing and stabilizing abilities, *Drug Dev. Ind. Pharm.,* 20, 2005, 1994.

(137) Partially methylated cyclodextrins and process for producing the same, Tsuchiyama, Y., Sato, M., Yagi, Y., and Ishikura, T., inventors, Sanraku Inc., assignee, US 4,746,734, May 24, 1988.

(138) **Harata, K.,** X-ray crystal structure of the permethylated ß-cyclodextrin complex with m-iodophenol. A stabilized skew-boat pyranose conformation in the distorted macrocyclic ring, *J. Chem. Soc. Chem. Commun.,* 928, 1988.

(139) **Harata, K., Uekama, K., Otagiri, M., and Hirayama, F.,** Conformation of permethylated cyclodextrins and the host–guest geometry of their inclusion complexes, *J. Inclusion Phenom.,* 1, 279, 1984.

(140) **Müller, B. W. and Brauns, U.,** Solubilization of drugs by modified beta-cyclodextrins, *Int. J. Pharm.,* 26, 77, 1985.

(141) **Yoshida, A., Yamamoto, M., Irie, T., Hirayama, F., and Uekama, K.,** Some pharmaceutical properties of 3-hydroxypropyl- and 2,3-dihydroxypropyl-ß-cyclodextrins and their solubilizing and stabilizing abilities, *Chem. Pharm. Bull.,* 37, 1059, 1989.

(142) Wasserlösliche Mischether des beta-cyclodextrins und ein Verfahren zu ihrer Herstellung: Water-soluble mixed ethers of ß-cyclodextrin and a process for their separation, Brandt, L. and Felcht, U., inventors, Hoeschst Aktiengessellschaft, assignee, DE 3345780, Dec 17, 1983.

(143) Phamazeutische Präparate von in Wasser schwerlöslichen oder instabilen Arzneistoffen und Verfahren zu ihrer Herstellung: Pharmaceutical compositions containing drugs which are unstable or sparingly soluble in water, and methods for their preparation, Brauns, U., and Müller, B. W. W., inventors, Janssen Pharmaceutica N. V., assignee, DE 3346123, December 21, 1983.

(144) Novel derivatives of gamma-cyclodextrin, Müller, B. W. W., inventor, Janssen Pharmaceutica N. V., assignee, GB 8506792, March 15, 1985. EP Application No. 86200334.0 4, March 1986.

(145) Water-soluble forms of retinoids, Pitha, J., inventor, United States Government, assignee, US 4,371,673, February 1, 1983.

(146) Administration of sex hormones in the form of hydrophilic cyclodextrin derivatives, Pitha, J., inventor, United States of America, Secretary, Dept. of Health and Human Services, assignee, US 4,596,795, June 24, 1986.

(147) Pharmaceutical preparations containing cyclodextrin derivatives, Pitha, J., inventor, United States of America, Department of Health and Human Services, assignee, US 4,727,064, February 23, 1988.

(148) **Müller, B. W. and Brauns, U.,** Hydroxypropyl-ß-cyclodextrin derivatives: Influence of average degree on substitution on complexing ability and surface activity, *J. Pharm. Sci.,* 75, 571, 1986.

(149) **Lindberg, B., Lindberg, J., Pitha, J., Rao, C. T., and Harata, K.,** Synthesis of some 2-O-(2-hydroxyalkyl) and 2-O-(2,3-dihydroxyalkyl) derivatives of cyclomaltoheptaose, *Carbohydr. Res.,* 222, 113, 1991.

(150) **Harata, K., Rao, C. T., and Pitha, J.,** Crystal structure of 6-O-[(R)-2-hydroxypropyl]cyclomaltoheptaose and 6-O-[(S)-2-hydroxypropyl]cyclomaltoheptaose, *Carbohydr. Res.,* 247, 83, 1993.

(151) **Müller, B. W., Brauns, U., and Backensfeld, T.,** Cyclodextrin derivatives for solubilization, stabilization, and absorption of drugs, in *Proceedings of the 4th International Symposium on Cyclodextrins,* Huber, O. and Szejtli, J., Eds., Kluwer Academic Publishers, Dordrecht, 1988.

(152) **Pitha, J.,** Amorphous water-soluble derivatives of cyclodextrins: From test tube to patient, *J. Controlled Rel.,* 6, 309, 1987.

(153) **Casu, B., Scovenna, G., Cifonelli, A. J., and Perlin, A. S.,** Infrared spectra of glycosaminoglycans in deuterium oxide and deuterium chloride solution, quantitative evaluation of uronic acid and acetamidodeoxyhexose moieties, *Carbohydr. Res.,* 63, 13, 1978.

(154) New catalytically oxidized polyglucosic units and methods of making same leading to aldehyde groups on the C-6 position of polyglucosic product and new products therefrom, Yalpani, M. and Abdel-Malik, M. M., inventors, Domtar, Inc., assignee, US 4,906,579, March 6, 1990.

(155) Cyclodextrins with anionic properties, Parmerter, S. M., Allen, Jr., E. E., and Hull, G. A., inventors, Corn Products Co., assignee. US. 3,426,011, February 4, 1969.

(156) **Terabe, S., Ozaki, H., Otsuka, K., and Ando, T.,** Electrokinetic chromatography with 2-O-carboxymethyl-ß-cyclodextrin as a moving "stationary" phase, *J. Chromatogr.,* 332, 1985.

(157) **Reuben, J., Rao, C. T., and Pitha, J.,** Distribution of substituents in carboxymethyl ethers of cyclomaltoheptaose, *Carbohydr. Res.,* 258, 281, 1994.

(158) **Uekama, K., Horiuchi, Y., Irie, T., and Hirayama, F.,** O-Carboxymethyl-O-ethylcyclomaltoheptaose as a delayed-release-type drug carrier: Improvement of the oral bioavailability of diltiazem in the dog, *Carbohydr. Res.,* 192, 323, 1989.

(159) **Uekama, K., Horikawa, T., Horiuchi, Y., and Hirayama, F.,** *In vitro* and *in vivo* evaluation of delayed-release behavior of diltiazem from its O-carboxymethyl-O-ethyl ß-cyclodextrin complex, *J. Controlled Rel.,* 25, 99, 1993.

(160) **Horiuchi, Y., Abe, K., Hirayama, F., and Uekama, K.,** Release control of theophylline by ß-cyclodextrin derivatives: Hybridizing effect of hydrophilic, hydrophobic, and ionizable ß-cyclodextrin complexes, *J. Controlled Rel.,* 15, 177, 1991.

(161) **Adachi, H., Irie, T., Uekama, K., Manako, T., Yano, T., and Saita, M.,** Combination effects of O-carboxymethyl-O-ethyl-ß-cyclodextrin and penetration enhancer HPE-101 on transdermal delivery of prostaglandin E1 in hairless mice, *Eur. J. Pharm. Sci.,* 1, 117, 1993.

(162) **Uekama, K., Adachi, H., Irie, T., Yano, T., Saita, M., and Noda, K.,** Improved transdermal delivery of prostaglandin E1 through hairless mouse skin: Combined use of carboxymethyl ethyl ß-cyclodextrin and penetration enhancers, *J. Pharm. Pharmacol.,* 44, 119, 1992.

(163) Cycloamylose sulfates and derivatives thereof, Bergeron, L. and Lee, L., inventors, US 2,923,704, 1960.

(164) **Pitha, J., Mallis, L. M., Lamb, D. J., Irie, T., and Uekama, K.,** Cyclodextrin sulfates: Characterization as polydisperse and amorphous mixtures, *Pharm. Res.,* 8, 1151, 1991.

(165) **Menger, F. M. and Williams, D. Y.,** Synthesis and properties of a surfactant-cyclodextrin conjugate, *Tetrahedron Lett.,* 27, 2579, 1986.

(166) **Lammers, J. N. J., Koole, J. L., and Hurkmans, J.,** Properties of cyclodextrins, VI. Water-soluble cyclodextrin derivatives, preparation and analysis, *Staerke,* 23, 167, 1971.

(167) Derivatives of cyclodextrins exhibiting enhanced aqueous solubility and the use thereof, Stella, V. and Rajewski, R., inventors, University of Kansas, assignee, WO. 9111172. A1. 8, August 1991.

(168) **Kano, K., Zhou, B., Sakaguchi, M., Matsumoto, H., and Hashimoto, S.,** Effects of aeration, freezing, and thawing on self-aggregation of naphthalene in aqueous beta-cyclodextrin solutions, *Sci. Eng. Rev. Doshisha Univ.,* 25, 253, 1985.

(169) **Gerloczy, A., Hoshino, T., and Pitha, J.,** Safety of oral cyclodextrins: Effects of (hydroxypropyl) cyclodextrins, cyclodextrin sulfates and cationic cyclodextrins on steroid balance in rats, *J. Pharm. Sci.,* 83, 193, 1994.

(170) **Okimoto, K., Rajewski, R. A., Jona, J. A., and Stella, V. J.,** The interaction of charged and uncharged drugs with a neutral (HP-ß-CD) and anionically charged (SBE7-ß-CD) ß-cyclodextrin, *Pharm. Res.,* 12, 205, 1995.

(171) **Johnson, M. D., Hoesterey, B. L., and Anderson, B. D.,** Solubilization of a tripeptide HIV protease inhibitor using a combination of ionization and complexation with chemically modified cyclodextrins, *J. Pharm. Sci.,* 83, 1142, 1994.

(172) **Rajewski, R. A., Traiger, G., Bresnahan, J., Jaberaboansari, P., Stella, V. J., and Thompson, D. O.,** Preliminary safety evaluation of parenterally administered sulfoalkyl ether ß-cyclodextrin derivatives, *J. Pharm. Sci.,* 84, 927, 1995.

(173) **Järvinen, K., Järvinen, T., Thompson, D. O., and Stella, V. J.,** The effect of a modified ß-cyclodextrin, SBE4-ß-CD, on the aqueous stability and ocular absorption of pilocarpine, *Curr. Eye Res.,* 13, 897, 1994.

(174) **Gorecka, B. A., Sanzgiri, Y. D., Bindra, D. S., and Stella, V. J.,** Effect of SBE4-ß-CD, a sulfobutyl ether ß-cyclodextrin, on the stability and solubility of O6-benzylguanine (NSC-637037) in aqueous solutions, *Int. J. Pharm.,* 125, 55, 1995.

(175) **Stella, V. J., Lee, H. K., and Thompson, D. O.,** The effect of SBE4-ß-CD on i.v. methylprednisolone pharmacokinetics in rats: Comparison to a co-solvent solution and two water-soluble prodrugs, *Int. J. Pharm.,* 120, 189, 1995.

(176) **Stella, V. J., Lee, H. K., and Thompson, D. O.,** The effect of SBE4-ß-CD on i.m. prednisolone pharmacokinetics and tissue damage in rabbits: Comparison to a co-solvent solution and a water-soluble prodrug, *Int. J. Pharm.,* 120, 197, 1995.

(177) **Järvinen, T., Järvinen, K., Urtti, A., Thompson, D., and Stella, V. J.,** Sulfobutyl ether ß-cyclodextrin (SBE-ß-CD) in eyedrops improves the tolerability of a topically applied pilocarpine prodrug in rabbits, *J. Ocular Pharm. Therapeut.,* 11, 95, 1995.

(178) **Järvinen, T., Järvinen, K., Schwarting, N., and Stella, V. J.,** ß-cyclodextrin derivatives, SBE4-ß-CD and HP-ß-CD, increase the oral bioavailability of cinnarizine in beagle dogs, *J. Pharm. Sci.,* 84, 295, 1995.

(179) **Olivier, P., Verwaerde, F., and Hedges, A. R.,** Subchronic toxicity of orally administered beta-cyclodextrin in rats, *J. Am. Coll. Toxicol.,* 10, 407, 1991.

(180) **Koizumi, K., Kubota, Y., Okada, Y., and Utamura, T.,** Microanalyses of beta-cyclodextrin in plasma by high-performance liquid chromatography, *J. Chromatogr.,* 341, 31, 1985.

(181) **Szejtli, J., Gerlóczy, A., and Fónagy, A.,** Intestinal absorption of carbon-14-labeled beta-cyclodextrin in rats, *Arzneim.-Forsch.,* 30, 808, 1980.

(182) **Gerlóczy, A., Fónagy, A., Keresztes, P., Perlaky, L., and Szejtli, J.,** Absorption, distribution, excretion, and metabolism of orally administered ^{14}C-beta-cyclodextrin in rat, *Arzneim.-Forsch.,* 35, 1042, 1985.

(183) **Szabo, P., Ferenczy, T., Serfözö, J., Szejtli, J., and Lipták, A.,** Absorption and elimination of cyclodextrin derivatives by rabbits and rats, in *Proceedings of the 1st International Symposium on Cyclodextrins,* Szjetli, J., Ed., Kluwer Academic Publishers, Dordrecht, 1982.

(184) **Irie, T., Tsunenari, Y., Uekama, K., and Pitha, J.,** Effect of bile on the intestinal absorption of α-cyclodextrin in rats, *Int. J. Pharm.,* 43, 41, 1988.

(185) **Miyajima, K., Yokoi, M., Komatsu, H., and Nakagaki, M.,** Interaction of ß-cyclodextrin with bile salts in aqueous solutions, *Chem. Pharm. Bull.,* 34, 1395, 1986.

(186) **Szejtli, J.,** The metabolism, toxicity, and biological effects of cyclodextrins, in *Cyclodextrins and Their Industrial Uses,* Duchêne D., Ed., Editions de Santé, Paris, 1987.

(187) **French, D.,** The Schardinger dextrins, *Adv. Carbohydr. Chem.,* 12, 189, 1957.

(188) **Mora, S., Simon, I., and Elödi, P.,** Active center of pancreatic amylase, I. Binding of beta-cyclodextrin, *Mol. Cell. Biochem.,* 4, 205, 1974.

(189) **Gerlóczy, A., Fónagy, A., and Szejtli, J.,** Absorption and metabolism of beta-cyclodextrin by rats, in *Proceedings of the 1st International Symposium on Cyclodextrins,* Szejtli, J., Ed., Kluwer Academic Publishers, Dordrecht, 1982.

(190) **Antenucci, R. N. and Palmer, J. K.,** Enzymic degradation of alpha- and beta-cyclodextrins by Bacteroides of the human colon, *J. Agric. Food Chem.,* 32, 1316, 1984.

(191) **Yoshimu, C., Sakurai, K., Omura, Y., Nobara, M., and Ando, T.,** Bifidobacterium-growth stimulant, *Ger. Offnen,* DE 3.205.018.

(192) **Suzuki, M. and Sato, A.,** Nutritional significance of cyclodextrins: Indigestibility and hypolipemic effect of alpha-cyclodextrin, *J. Nutr. Sci. Vitaminol.,* 31, 209, 1985.

(193) **Flourié, B., Molis, C., Achour, L., Dupas, H., Hatat, C., and Rambaud, J. C.,** Fate of ß-cyclodextrin in the human intestine, *J. Nutr.,* 123, 676, 1993.

(194) **Anderson, G. H., Robbins, F. M., Domingues, F. J., Moores, G. R., and Long, C. L.,** The utilization of Schardinger dextrins by the rat, *Toxicol. Appl. Pharmacol.,* 5, 257, 1962.

(195) **Szejtli, J.,** in *Cyclodextrins and Their Inclusion Complexes,* Akadémiai Kiadó, Budapest, 1982, 41.

(196) **Szejtli, J. and Sebestyén, G.,** Resorption, metabolism, and toxicity studies on the peroral application of beta-cyclodextrin, *Starch/Staerke,* 31, 385, 1979.

(197) **Makita, T., Ojima, N., Hashimoto, Y., Ide, H., Tsuji, M., and Fujisaki, Y.,** Chronic oral toxicity study of beta-cyclodextrine (beta-CD) in rats, *Oyo Yakuri,* 10, 449, 1975.

(198) **Gergely, V., Sebestyén, G., and Virág, S.,** Toxicity studies of ß-cyclodextrin, in *Proceedings of the 1st International Symposium on Cyclodextrins,* Szjetli, J., Ed., Kluwer Academic Publishers, Dordrecht, 1982.

(199) **Bellringer, M. E., Smith, T. G., Read, R., Gopinath, C., and Olivier, P.,** ß-cyclodextrin: 52-week toxicity studies in the rat and dog, *Food Chem. Toxicol.,* 33, 367, 1995.

(200) **Reiser, S., and Lewis, C. G.,** Effect of the type of dietary carbohydrate on small intestinal functions, in *Metabolic Effects of Dietary Carbohydrates,* London and Vrane A., Prague, 1986.

(201) **Mackenzie, L. M. and Hauck, W. M.,** Three general reproduction studies in rats ingesting up to 10% sorbitol in the diet: A brief review of the toxicological status of sorbitol in food. *Food Chem. Toxicol.,* 191, 1986.

(202) **Irie, T., Fukunaga, K., and Pitha, J.,** Hydroxypropyl cyclodextrins in parenteral use, I. Lipid dissolution and effects on lipid transfers *in vitro, J. Pharm. Sci.,* 81, 521, 1992.

(203) **Frijlink, H. W., Eissens, A. C., Hefting, N. R., Poelstra, K., Lerk, C. F., and Meijer, D. K. F.,** The effect of parenterally administered cyclodextrins on cholesterol levels in the rat, *Pharm. Res.,* 8, 9, 1991.

(204) **Manabe, M., Kwamura, H., Morita, H., and Ikushima, K.,** Determination of binding constant for cyclodextrin with bile salt by the differential conductivity method, in *7th International Cyclodextrin Symposium Proceedings,* Osa, T., Hashimoto, H., Hattori, K., Hirai, H., Komiyama, M., Nagai, T., Nakamura, N., Takahashi, K., Takahashi, T., Takayama, K., Toda, F., Ueda, H., Ueno, A., and Uekama, K., Eds., Business Center for Academic Societies, Tokyo, 1994.

(205) **Tan, X. and Lindenbaum, S.,** Studies on complexation between ß-cyclodextrin and bile salts, *Int. J. Pharm.,* 74, 127, 1991.

(206) **Tan, Z. J., Zhu, X. X., and Brown, G. R.,** Formation of inclusion complexes of cyclodextrins with bile salt anions as determined by NMR titration studies, *Langmuir,* 10, 1034, 1994.

(207) **Riottot, M., Olivier, P., Huet, A., Caboche, J. J., Parquet, M., Khallou, J., and Lutton, C.,** Hypolipidemic effects of ß-cyclodextrin in the hamster and in the genetically hypercholesterolemic Rico rat, *Lipids,* 28, 181, 1993.

(208) **Levrat, M. A., Favier, M. L., Moundras, C., Rémésy, C., Demigné, C., and Morand, C.,** Role of dietary propionic acid and bile acid excretion in the hypocholesterolemic effects of oligosaccharides in rats, *J. Nutr.,* 124, 531, 1994.

(209) **Moundras, C., Behr, S. R., Demigné, C., Mazur, A., and Rémésy, C.,** Fermentable polysaccharides that enhance fecal bile acid excretion lower plasma cholesterol and apolipoprotein E-rich HDL in rats, *J. Nutr.,* 124, 2179, 1994.

(210) **Abadie, C., Hug, M., Kubli, C., and Gains, N.,** Effect of cyclodextrins and undigested starch on the loss of chenodeoxycholate in the feces, *Biochem. J.,* 299, 725, 1994.

(211) **Comini, S., Olivier, P., Riottot, M., and Duhamel, D.,** Interaction of ß-cyclodextrin with bile acids and their competition with vitamins A and D3 as determined by 1H-NMR spectrometry, *Clin. Chim. Acta,* 228, 181, 1994.

(212) **Antlsperger, G.,** New aspects in cyclodextrin toxicology, in *Mins 6th International Symposium on Cyclodextrins,* Hedges, A. R., Ed., Editions de Santé, Paris, 1992.

(213) **Hiasa, Y., Ohshima, M., Kitahori, Y., Konishi, N., Fujita, T., and Yuasa, T.,** Beta-cyclodextrin: Promoting effect on the development of renal tubular cell tumors in rats treated with N-ethyl-N-hydroxyethylnitrosamine, *J. Natl. Cancer Inst.,* 69, 963, 1982.

(214) **Tabata, S., Ozono, S., Yamaguchi, H., Kitagawa, H., Uemura, H., Matsuki, H., Samma, S., Hirao, Y., Okajima, E., Kitahori, Y., and Hiasa, Y.,** Induction of nephrosis by ß-cyclodextrin in beagles, *J. Toxicol. Pathol.,* 4, 67, 1991.

(215) **Hiasa, Y., Konishi, N., Nakaoka, S., Nakamura, M., Nishii, S., Kitahori, Y., and Ohshima, M.,** Possible application to medium-term organ bioassays for renal carcinogenesis

modifiers in rats treated with N-ethyl-N- hydroxyethylnitrosamine and unilateral nephrectomy, *Jpn. J. Cancer Res.,* 82, 1385, 1991.

(216) **Hoffmann, A., Romen, W., and Neumann, H. G.,** Tumors in rat kidney generated by initiation with trans-4-acetylaminostilbene and several promoting treatments, *J. Cancer Res. Clin. Oncol.,* 119, 329, 1993.

(217) **Barrow, P. C., Olivier, P., and Marzin, D.,** The reproductive and developmental toxicity profile of beta-cyclodextrin in rodents, *Reprod. Toxicol.,* 9, 389, 1995.

(218) *FDA Guideline for Industry Dose Selection for Carcinogenicity Studies of Pharmaceuticals,* (available via ftp at cdvs.cder.fda.gov. [User Name = Anonymous, Password = your e-mail address] in the guidance subdirectory, ICHS1C.W60 = filename).

(219) **Frank, D. W., Gray, J. E., and Weaver, R. N.,** Cyclodextrin nephrosis in the rat, *Am. J. Pathol.,* 83, 367, 1976.

(220) **Diomi, P., Ericsson, J. L. E., Matheson, N. A., and Shearer, J. R.,** Studies on renal tubular morphology and toxicity after large doses of dextran 40 in the rabbit, *Lab Invest.,* 22, 355, 1970.

(221) **Janigan, D. T. and Santamaria, A.,** A histochemical study of swelling and vacuolation of proximal tubular cells in sucrose nephrosis in the rat, *Am. J. Pathol.,* 39, 175, 1961.

(222) **Maunsbach, A. B., Madden, S. C., and Latta, H.,** Light and electron microscopic changes in proximal tubules of rats after administration of glucose, mannitol, sucrose, or dextran, *Lab Invest.,* 11, 421, 1962.

(223) **Trump, B. F. and Janigan, D. T.,** The pathogenesis of cytologic vacuolization in sucrose nepthrosis: An electron microscopic and histochemical study, *Lab Invest.,* 11, 395, 1962.

(224) **Serfözö, J. and Tôth-Jakab, A.,** Effect of beta-cyclodextrin on the epithelial cells of the proximal tubules in rabbit kidney, *Acta Biol. Debrecina,* 19, 87, 1984.

(225) **Hiasa, Y., Oshima, M., Kitahori, Y., Yuasa, T., Fujita, T., Iwata, C., Miyashiro, A., and Konishi, N.,** Histochemical studies of ß-cyclodextrin nephrosis in rats, *J. Nara Med. Assoc.,* 32, 316, 1981.

(226) **Sharma, A. and Janis, L. S.,** Lipoprotein-cyclodextrin interaction, *Clin. Chim. Acta,* 199, 129, 1991.

(227) **Frijlink, H. W., Visser, J., Hefting, N. R., Oosting, R., Meijer, D. K. F., and Lerk, C. F.,** The pharmacokinetics of ß-cyclodextrin and hydroxypropyl-ß-cyclodextrin in the rat, *Pharm. Res.,* 7, 1248, 1990.

(228) **Matsuda, K., Mera, Y., Segawa, Y., Uchida, I., Yokomine, A., and Takagi, K.,** Acute toxicity study of gamma-cyclodextrin (gamma-CD) in mice and rats, *Oyo Yakuri,* 26, 287, 1983.

(229) **Schmid, G.,** Preparation and application of γ-cyclodextrin, in *New Trends in Cyclodextrins and Derivatives,* Duchêne D., Ed., Editions de Santé, Paris, 1991.

(230) **Irie, T., Otagiri, M., Sunada, M., Uekama, K., Ohtani, Y., Yamada, Y., and Sugiyama, Y.,** Cyclodextrin-induced hemolysis and shape changes of human erythrocytes *in vitro, J. Pharmacobio-Dyn.,* 5, 741, 1982.

(231) **Pitha, J., Irie, T., Sklar, P. B., and Nye, J. S.,** Drug solubilizers to aid pharmacologists: Amorphous cyclodextrin derivatives, *Life Sci.,* 43, 493, 1988.

(232) **Garay, R. P., Féray, J. C., Fanous, K., Nazaret, C., Villegas, M. J., and Letavernier, J. F.,** A new approach for the *in vitro* evaluation of cyclodextrin effects on cellular membranes of human cells, in *7th International Cyclodextrin Symposium Proceedings,* Osa, T., Hashim-

oto, H., Hattori, K., Hirai, H., Komiyama, M., Nagai, T., Nakamura, N., Takahashi, K., Takahashi, T., Takayama, K., Toda, F., Ueda, H., Ueno, A., and Uekama, K., Eds., Business Center for Academic Societies, Tokyo, 1994.

(233) **Leroy-Lechat, F., Wouessidjewe, D., Andreux, J. P., Puisieux, F., and Duchêne, D.,** Evaluation of the cytotoxicity of cyclodextrins and hydroxypropylated derivatives, *Int. J. Pharm.*, 101, 97, 1994.

(234) **Bar, R. and Ulitzur, S.,** Bacterial toxicity of cyclodextrins: Luminous Escherichia coli as a model, *Appl. Microbiol. Biotechnol.*, 41, 574, 1994.

(235) **Miyajima, K., Saito, H., and Nakagaki, M.,** Destruction of liposomes by cyclodextrins, *J. Pharmacobio-Dyn.*, 10, s-123, 1987.

(236) **Ohtani, Y., Irie, T., Uekama, K., Fukunaga, K., and Pitha, J.,** Differential effects of α-, ß- and γ-cyclodextrins on human erythrocytes, *Eur. J. Biochem.*, 186, 17, 1989.

(237) **Nakanishi, K., Nadai, T., Masada, M., and Miyajima, K.,** Effect of cyclodextrins on biological membrane, II. Mechanism of enhancement on the intestinal absorption of non-absorbable drug by cyclodextrins, *Chem. Pharm. Bull.*, 40, 1252, 1992.

(238) **Irie, T., Wakamatsu, K., Arima, H., Aritomi, H., and Uekama, K.,** Enhancing effects of cyclodextrins on nasal absorption of insulin in rats, *Int. J. Pharm.*, 84, 129, 1992.

(239) **Frömming, K. H., Fridrich, R., and Mehnert, W.,** Inclusion compounds of cholesterol and ß-cyclodextrin, *Eur. J. Pharm. Biopharm.*, 39, 148, 1993.

(240) **Fridrich, R., Mehnert, W., and Frömming, K. H.,** Studies of the inclusion compound between ß-cyclodextrin and cholesterol, in *Mins 5th International Cyclodextrin Symposium,* Duchêne D., Ed., Editions de Santé, Paris, 1990.

(241) **Claudy, P., Letoffe, J. M., Germain, P., Bastide, J. P., Bayol, A., Blasquez, S., Rao, R. C., and Gonzalez, B.,** Physicochemical characterization of cholesterol-beta cyclodextrin inclusion complexes, *J. Therm. Anal.*, 37, 2497, 1991.

(242) **Svendsen, O.,** ß-cyclodextrin and local muscle toxicity of intramuscular drug formulations, *Target Organ Toxic Process Arch. Toxicol.*, Suppl., 12, 391, 1988.

(243) **Szabo, P., Ferenczy, T., Serfözö, J., and Szejtli, J.,** Investigation of the absorption and elimination conditions of beta-cyclodextrin and its derivatives, in *Biochem. Metab. Sci. Mater. Pap. Colloq.*, Klebovich, I., Laszlovszky, I., and Rosdy, B., Eds., Hungarian Chemistry Society Biochemistry Section, Akadémiai Kiadó, Budapest, 1981.

(244) **Szatmári, I. and Vargay, Z.,** Pharmacokinetics of dimethyl-beta-cyclodextrin in rats, in *Proceedings of the 4th International Symposium on Cyclodextrins,* Duchêne D., Ed., Kluwer Academic Publishers, Dordrecht, 1988.

(245) **Serfözö, J., Szabo, P., Ferenczy, T., and Tóth-Jakab, A.,** Renal effects of parenterally administered methylated cyclodextrins on rabbits, in *Proceedings of the 1st International Symposium on Cyclodextrins,* Szejtli, J., Ed., Kluwer Academic Publishers, Dordrecht, 1982.

(246) **Yamamoto, M., Aritomi, H., Irie, T., Hirayama, F., and Uekama, K.,** Pharmaceutical evaluation of branched ß-cyclodextrins as parenteral drug carriers, in *Mins. 5th International Symposium on Cyclodextrins,* Duchêne D., Ed., Editions de Santé, Paris, 1990.

(247) **Jodál, I., Nánási, P., and Szejtli, J.,** Investigation of the hemolytic effect of cyclodextrin derivatives, in *Proceedings of the 4th International Symposium on Cyclodextrins,* Huber, O. and Szejtli, J., Eds., Kluwer Academic Publishers, Dordrecht, 1988.

(248) **Yoshida, A., Arima, H., Uekama, K., and Pitha, J.,** Pharmaceutical evaluation of hydroxyalkyl ethers of ß-cyclodextrins, *Int. J. Pharm.*, 46, 217, 1988.

(249) **Hovgaard, L. and Broendsted, H.,** A study on the effect of cyclodextrins, a new class of absorption enhancers, on CACO-2 cell monolayers, in *Proceedings of the 20th International Symposium on Controlled Release Bioact Mater,* Roseman, T. J., Peppas, N. A., and Gabelnick, H. L., Eds., Controlled Release Society, Deerfield, Illinois, 1993.

(250) **Agerholm, C., Bastholm, L., Johansen, P. B., Nielsen, M. H., and Elling, F.,** Epithelial transport and bioavailability of intranasally administered human growth hormone formulated with the absorption enhancers didecanoyl-L-α-phosphatidylcholine and α-cyclodextrin in rabbits, *J. Pharm. Sci.*, 83, 1706, 1994.

(251) **Nakai, Y., Yamamoto, K., Terada, K., Horibe, H., and Ozawa, K.,** Interaction of tri-O-methyl-beta-cyclodextrin with drugs, II. Enhanced bioavailability of ketoprofen in rats when administered with tri-O-methyl-beta-cyclodextrin, *Chem. Pharm. Bull.*, 31, 3745, 1983.

(252) **Kikuchi, M., Hirayama, F., and Uekama, K.,** Improvement of oral and rectal bioavailabilities of carmofur by methylated ß-cyclodextrin complexations, *Int. J. Pharm.*, 38, 191, 1987.

(253) **Arima, H., Kondo, T., Irie, T., and Uekama, K.,** Enhanced rectal absorption and reduced local irritation of the anti-inflammatory drug ethyl 4-biphenylylacetate in rats by complexation with water-soluble ß-cyclodextrin derivatives and formulation as oleaginous suppository, *J. Pharm. Sci.*, 81, 1119, 1992.

(254) **Okamoto, H., Komatsu, H., Hashida, M., and Sezaki, H.,** Effects of ß-cyclodextrin and di-O-methyl-ß-cyclodextrin on the percutaneous absorption of butylparaben, indomethacin, and sulfanilic acid, *Int. J. Pharm.*, 30, 35, 1986.

(255) **Hermens, W. A. J., Deurloo, M. J. M., Romeyn, S. G., Verhoef, J. C., and Merkus, F. W. H.,** Nasal absorption enhancement of 17ß-estradiol by dimethyl-ß-cyclodextrin in rabbits and rats, *Pharm. Res.*, 7, 500, 1990.

(256) **Schipper, N. G. M., Hermens, W. A. J., Romeyn, S. G., Verhoef, J., and Merkus, F. W. H.,** Nasal absorption of 17ß-estradiol and progesterone from a dimethyl cyclodextrin inclusion formulation in rats, *Int. J. Pharm.*, 64, 61, 1990.

(257) **Watanabe, Y., Matsumoto, Y., Kawamoto, K., Yazawa, S., and Matsumoto, M.,** Absorption enhancement to polypeptide drugs by cyclodextrins, II. Enhancing effect of cyclodextrins on nasal absorption of insulin and its duration in rabbits, *Chem. Pharm. Bull.*, 40, 3100, 1992.

(258) **Gerlóczy, A., Antal, S., Szatmari, I., Müller-Horvath, R., and Szejtli, J.,** Absorption, distribution, and excretion of carbon-14 labeled hydroxypropyl ß-cyclodextrain in rats following oral administration, in *Mins. 5th International Symposium on Cyclodextrins,* Duchêne D., Ed., Editions de Santé, Paris, 1990.

(259) **Monbaliu, J., Van Beijsterveldt, L., Meuldermans, W., Szathmary, S., and Heykants, J.,** Disposition of hydroxypropyl ß-cyclodextrin in experimental animals, in *Mins 5th International Symposium on Cyclodextrins,* Duchêne D., Ed., Editions de Santé, Paris, 1990.

(260) **Szathmary, S. C., Seiler, K. U., Luhmann, I., and Huss, H. J.,** Pharmacokinetic behavior and absolute bioavailability of hydroxypropyl ß-cyclodextrin after increasing doses in volunteers, in *Mins. 5th International Symposium on Cyclodextrins,* Duchêne D., Ed., Editions de Santé, Paris, 1990.

(261) **Coussement, W., Van Cauteren, H., Vandenberghe, J., Vanparys, P., Teuns, G., Lampo, A., and Marsboom, R.,** Toxicological profile of hydroxypropyl ß-cyclodextrin (HP ß-

CD) in laboratory animals, in *Mins. 5th International Symposium on Cyclodextrins*, Duchêne D., Ed., Editions de Santé, Paris, 1990.
(262) **Brewster, M. E. and Bodor, N.,** Parenteral safety and uses of 2-hydroxypropyl ß-cyclodextrin, in *Mins. 5th International Symposium on Cyclodextrins*, Duchêne D., Ed., Editions de Santé, Paris, 1990.
(263) **Van Cauleren, H., Lampo, A., Lammens, L., Benze, H., Coussement, W., and Vandenberghe, J.,** Monitoring pharmacodynamic effects in toxicology studies, in *Workshop on the Use of Pharmacology Studies in Drug Safety Assessment—Present Situation and Future Perspectives*, Sundwall, A., Johansson, B., Lindbom, L., Lindgren E., and Sjöberg, P., Eds., Sweden, 1994.
(264) **Patten, J. R., Richards, E. A., and Pope, H.,** The effect of raw soyabean on the pancreas of adult dogs, *Proceedings of the Society for Experimental Biology and Medicine*, Cambridge, 1971, 37.
(265) **Rackis, J. J.,** Physiological properties of soyabean trypsin inhibitors and their relationship to pancreatic hypertrophy and growth inhibition of rats, *Federation Proceedings*, 24, 1488, 1965.
(266) **Crass, R. A. and Morgan, R. C. H.,** Effects of long-term feeding of soya-bean flour diets on pancreatic growth in the rat, *Brit. J. Nutr.*, 47, 119, 1982.
(267) **Aursnes, I.,** Drugs affecting lipid metabolism, in *Meyler's Side Effects of Drugs*, Dukes, M. N., Ed., Elsevier Amsterdam, London, 1992.
(268) **Koop, I., Lindenthal, M., Schade, M., Trautmann, M., Adler, G., and Arnold, R.,** Role of cholecystokinin in cholestyramine-induced changes in the exocrine pancreas, *Pancreas*, 6, 564, 1991.
(269) **Kogire, M., Gomez, G., Uchida, T., Ishizuka, J., Greeley, Jr., G. H., and Thompson, J. C.,** Chronic effects of oral cholestyramine, a bile salt sequestrant, and exogenous cholecystokinin on insulin release in rats, *Pancreas*, 7, 15, 1992.
(270) **De Caprio, J., Yun, J., and Javitt, N. B.,** Bile acid and sterol solubilization in 2-hydroxypropyl ß-cyclodextrin, *J. Lipid Res.*, 33, 441, 1992.
(271) **Sundaram, S. and Dayan, A. D.,** Effects of a cholecystokinin receptor antagonist on rat exocrine pancreatic response to raw soya flour, *Human and Exp. Toxicol.*, 10, 179, 1991.
(272) **Seiler, K. U., Szathmary, S., Huss, H. J., De Coster, R., and Junge, W.,** Safety profile and intravenous tolerance of hydroxypropyl ß-cyclodextrin after increasing single dose, in *Mins. 5th International Symposium on Cyclodextrins*, Duchêne D., Ed., Editions de Santé, Paris, 1990.
(273) **Anderson, W. R., Calderwood-Mays, M., Brewster, M. E., and Bodor, N.,** The effects of chemically-modified cyclodextrins on the renal histology of the Sprague-Dawley rat, in *Mins. 6th International Symposium on Cyclodextrins*, Hedges, A. R., Ed., Editions de Santé, Paris, 1992.
(274) **Pitha, J. and Pitha, J.,** Amorphous water-soluble derivatives of cyclodextrins: Nontoxic dissolution enhancing excipients, *J. Pharm. Sci.*, 74, 987, 1985.
(275) **Brewster, M. E., Estes, K. S., and Bodor, N.,** An intravenous toxicity study of 2-hydroxypropyl-ß-cyclodextrin, a useful drug solubilizer, in rats and monkeys, *Int. J. Pharm.*, 59, 231, 1990.
(276) Cyclodextrin sulfate salts as complement inhibitors, Bernstein, S., Joseph, J. P., and Nair, V., inventors, American Cyanamid Co., assignee, US 4,020,160, April 26, 1977.

(277) Modified cyclodextrin sulfate salts as complement inhibitors, Lewis, A. J. and Bernstein, S., inventors, American Cyanamid Co., assignee, US 4,247,535, January 27, 1981.
(278) C6-modified cyclodextrin sulfate salts as complement inhibitors, Lewis, A. J. and Bernstein, S., inventors, American Cyanamid Co., assignee. US 4,25818, March 24, 1981.
(279) **Anand, R., Nayyar, S., Pitha, J., and Merril, C. R.,** Sulfated sugar alpha-cyclodextrin sulfate, a uniquely potent anti-HIV agent, also exhibits marked synergism with AZT, and lymphoproliferative activity, *Antiviral Chem.*, 1, 41, 1990.
(280) **Moriya, T., Kurita, H., Matsumoto, K., Otake, T., Mori, H., Morimoto, M., Ueba, N., and Kunita, N.,** Potent inhibitory effect of a series of modified cyclodextrin sulfates on the replication of HIV-1 *in vitro*, *J. Med. Chem.*, 34, 2301, 1991.
(281) **Schols, D., De Clercq, E., Witvrouw, M., Nakashima, H., Snoeck, R., Pauwels, R., Van Schepdael, A., and Claes, P.,** Sulfated cyclodextrins are potent anti-HIV agents acting synergistically with 2′,3′-dideoxynucleoside analogs, *Antiviral Chem.*, 2, 45, 1991.
(282) **Weiner, D. B., Williams, W. V., Weisz, P. B., and Greene, M. I.,** Synthetic cyclodextrin derivatives inhibit HIV infection *in vitro*, *Pathobiology*, 60, 206, 1992.
(283) **Moriya, T., Saito, K., Kurita, H., Matsumoto, K., Otake, T., Mori, H., Morimoto, M., Ueba, N., and Kunita, N.,** A new candidate for an anti-HIV-1 agent: Modified cyclodextrin sulfate (mCDS71), *J. Med. Chem.*, 36, 1674, 1993.
(284) **Otake, T., Schols, D., Witvrouw, M., Naesens, L., Nakashima, H., Moriya, T., Kurita, H., Matsumoto, K., Ueba, N., and De Clercq E.,** Modified cyclodextrin sulfates (mCDS11) have potent inhibitory activity against HIV and high oral bioavailability, *Antiviral Chem. Chemother.*, 5, 155, 1994.
(285) **Neyts, J., Snoeck, R., Schols, D., Balzarini, J., Esko, J. D., Van Schepdael, A., and De Clercq, E.,** Sulfated polymers inhibit the interaction of human cytomegalovirus with cell surface heparan sulfate, *Virology*, 189, 48, 1992.
(286) **Folkman, J., Weisz, P. B., Joullie, M. M., Li, W. W., and Ewing, W. R.,** Control of angiogenesis with synthetic heparin substitutes, *Science*, 243, 1490, 1989.
(287) **Stokes, C. L., Weisz, P. B., Williams, S. K., and Lauffenburger, D. A.,** Inhibition of microvascular endothelial cell migration by ß-cyclodextrin tetradecasulfate and hydrocortisone, *Microvasc. Res.*, 40, 279, 1990.
(288) **Li, W. W., Casey, R., Gonzalez, E. M., and Folkman, J.,** Angiostatic steroids potentiated by sulfated cyclodextrins inhibit corneal neovascularization, *Invest. Ophthalmol. Visual Sci.*, 32, 2898, 1991.
(289) **Skubitz, K. M. and Ehresmann, D. D.,** The angiogenesis inhibitor ß-cyclodextrin tetradecasulfate inhibits ecto-protein kinase activity, *Cell. Mol. Biol. (Oxford)*, 38, 543, 1992.
(290) **Teicher, B. A., Sotomayor, E. A., Huang, Z. D., Ara, G., Holden, S., Khandekar, V., and Chen, Y.,** ß-cyclodextrin tetradecasulfate/tetrahydrocortisol ± minocycline as modulators of cancer therapies *in vitro* and *in vivo* against primary and metastatic Lewis lung carcinoma, *Cancer Chemother. Pharmacol.*, 33, 229, 1993.
(291) **Proia, A. D., Hirakata, A., McInnes, J. S., Scroggs, M. W., and Parikh, I.,** The effect of angiostatic steroids and ß-cyclodextrin tetradecasulfate on corneal neovascularization in the rat, *Exp. Eye Res.*, 57, 693, 1993.
(292) **Wong, J., Wang, N., Miller, J. W., and Schuman, J. S.,** Modulation of human fibroblast activity by selected angiogenesis inhibitors, *Exp. Eye Res.*, 58, 439, 1994.

(293) **Herrmann, H. C., Okada, S. S., Hozakowska, E., LeVeen, R. F., Golden, M. A., Tomaszewski, J. E., Weisz, P. B., and Barnathan, E. S.,** Inhibition of smooth muscle cell proliferation and experimental angioplasty restenosis by ß-cyclodextrin tetradecasulfate, *Arterioscler. Thromb.*, 13, 924, 1993.

(294) **Bortolus, P., Grabner, G., Koehler, G., and Monti, S.,** Photochemistry of cyclodextrin host–guest complexes, *Coord. Chem. Rev.*, 125, 261, 1993.

(295) **Macarak, E. J., Kumor, K., and Weisz, P. B.,** Sulfation and hemolytic activity of cyclodextrin, *Biochem. Pharmacol.*, 42, 1502, 1991.

(296) **Shiotani, K., Uehata, K., Irie, T., Uekama, K., Thompson, D. O., and Stella, V. J.,** Differential effects of sulfate and sulfobutyl either of ß-cyclodextrin on erythrocyte membranes *in vitro*, *Pharm. Res.*, 12, 78, 1995.

(297) **Speid, L. H., Lumley, C., E., and Walker, S. R.,** Harmonization of guidelines for toxicity testing of pharmaceuticals by 1992, *Regulat. Toxicol. Pharmacol.*, 12, 179, 1990.

(298) **Enters, E. K.,** Regulatory considerations: Present and future, in *Pharmaceutical Excipients: Toward the Twenty-first Century*, Technolomic Publishing, Lancaster, Pennsylvania, 1995.

(299) *Pharmaceutical Technology Europe*, Kan, A., Ed., Advanstar Communications, Chester, England, 1993.

(300) **Cohen, L. I.,** The regulation of pharmaceutical excipients in Japan, *Pharm. Technol.*, 140, 1993.

(301) **Möller, H. and Oeser, W. H. E.,** *Drug Master Files: Global Hamonization of Quality Standards*, Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH, 1992.

(302) 21 Code of Federal Regulations 182.1–184.1.

(303) United States Pharmacopeia 23 National Formulary 18 Supplement 2, *United States Pharmacopeia National Formulary*, 23 & 18, Rockville, Maryland, 1994, 2595.

(304) 59 Federal Register 2413, January 15, 1994.

(305) **Steffan, B., Fischer, W., Cordes, G., Habon, I., and Mueller, R.,** Proton nuclear magnetic resonance (NMR) studies on the inclusion complex of prostaglandin E1 (PGE1) with alpha-cyclodextrin, *Pharm. Res.*, 9, 575, 1992.

(306) **Szente, L.,** Cyclodextrins: Structure, application, stability and their influence on the stability of active substances, *Paperback APV*, 32, 225, 1993.

(307) **Darrouzet, H.,** Practical considerations concerning the use of cyclodextrins as complexing agents, in *Mins. 6th International Symposium on Cyclodextrins*, Hedges, A. R., Ed., Editions de Santé, Paris, 1992.

(308) **Uekama, K.,** Cyclodextrins as high-performance materials, *Pharm. Technol. Jpn.*, 11, 331, 1995.

(309) Drug/CD complexes marketed in Japan, in *Cyclodextrin News*, Cyclolab, Budapest, 1995.

(310) **Jodal, I., Kandra, L., Harangi, J., Nanasi, P., and Szejtli, J.,** Hydrolysis of cyclodextrin by Aspergillus oryzae alpha-amylase, *Starch/Staerke*, 36, 140, 1984.

(311) **Abdel-Rahman, A. A., Saleh, S. I., Nakai, Y., Aboutaleb, A. E., and Ahmed M. O.,** Investigation of the interaction of bromazepam with cyclodextrins in solutions and in ground mixtures, *J. Pharm. Belg.*, 49, 23, 1994.

(312) **Al-Meshal, M. A., El-Mahrook, G. M., Al-Angary, A. A., and Gouda, M. W.,** Interaction of carbamazepine with cyclodextrins, *Pharm. Indust.*, 55, 1129, 1993.

(313) **Utsuki, T., Imamura, K., Hirayama, F., and Uekama, K.,** Stoichiometry-dependent changes of solubility and photoreactivity of an antiulcer agent, 2′-carboxymethoxy-4,4′-bis(3-methyl-2-butenyloxy)chalcone, in cyclodextrin inclusion complexes, *Eur. J. Pharm. Sci.*, 1, 81, 1993.

(314) **Nakai, Y., Aboutaleb, A. E., Yamamoto, K., Saleh, S. I., and Ahmed, M. O.,** Study of the interaction of clobazam with cyclodextrins in solution and in the solid state, *Chem. Pharm. Bull.*, 38, 728, 1990.

(315) **Helm, H., Andersen, A., Müller, B. W., and Waaler, T.,** Complex formation of ß- and γ-cyclodextrins with digitoxin, *Acta Pharm. Nord.*, 4, 313, 1992.

(316) **Ueda, H. and Perrin, J. H.,** A microcalorimetric investigation of the binding of flurbiprofen to cyclodextrins, *J. Pharm. Biomed. Anal.*, 4, 107, 1986.

(317) **Kearney, A. S., Mehta, S. C., and Radebaugh, G. W.,** The effect of cyclodextrins on the rate of intramolecular lactamization of gabapentin in aqueous solution, *Int. J. Pharm.*, 78, 25, 1992.

(318) **Torres-Labandeira, J. J., Echezarreta-López, M., Santana-Penín, L., and Vila-Jato, J. L.,** Glibornuride-ß-cyclodextrin inclusion complexes, preparation, structural characterization, and *in vitro* dissolution behavior, *Eur. J. Pharm. Biopharm.*, 39, 255, 1993.

(319) **Mazzi, G., Vincieri, F. F., Forni, F., Mulinacci, N., and Celli, S.,** Formation of inclusion complex between the nonsteroidal antiinflammatory drug (RS)-2-(4-isobutylphenyl)propiohydroxamic acid and ß-cyclodextrin, *Acta Pharm. Technol.*, 34, 17, 1988.

(320) **Han, S. M., Purdie, N., and Swallows, K. A.,** Determination of the benzodiazepin-2-ones by circular dichroism, *Anal. Chim. Acta*, 197, 57, 1987.

(321) **Menard, F. A., Dedhiya, M. G., and Rhodes, C. T.,** Studies of the effect of pH, temperature, and ring size on the complexation of phenytoin with cyclodextrins, *Pharm. Acta Helv.*, 63, 303, 1988.

(322) **Cabral Marques, H. M., Hadgraft, J., and Kellaway, I. W.,** Studies of cyclodextrin inclusion complexes, I. The salbutamol-cyclodextrin complex as studied by phase solubility and DSC., *Int. J. Pharm.*, 63, 259, 1990.

(323) **Yusuff, N. and York, P.,** Spironolactone-cyclodextrin complexes: Phase solubility and ultrafiltration studies, *Int. J. Pharm.*, 73, 9, 1991.

(324) **Szeman, J., Fenyvesi, E., Szejtli, J., Ueda, H., Machida, Y., and Nagai, T.,** Water soluble cyclodextrin polymers: Their interaction with drugs, *J. Inclusion Phenom.*, 5, 427, 1987.

(325) **Green, A. R. and Guillory, J. K.,** Heptakis(2,6-di-O-methyl)-ß-cyclodextrin complexation with the antitumor agent chlorambucil, *J. Pharm. Sci.*, 78, 427, 1989.

(326) **Abdel Rahman, A. A., Saleh, S. I., Nakai, Y., Aboutaleb, A. E., and Ahmed, M. O.,** Molecular behavior and dissolution characteristics of flunitrazepam in ground mixtures with cyclodextrins, *Eur. J. Pharm. Biopharm.*, 39, 212, 1993.

(327) **Acarturk, F., Imai, T., Saito, H., Ishikawa, M., and Otagiri, M.,** Comparative study on inclusion complexation of maltosyl-ß-cyclodextrin, heptakis(2,6-di-O-methyl)-ß-cyclodextrin and ß-cyclodextrin with fucosterol in aqueous and solid state, *J. Pharm. Pharmacol.*, 45, 1028, 1993.

(328) **Imai, T., Irie, T., Otagiri, M., Uekama, K., and Yamasaki, M.,** Comparative study on inclusion complexations of antiinflammatory drug flurbiprofen with beta-cyclodextrin and methylated beta-cyclodextrins, *J. Inclusion Phenom.*, 2, 597, 1984.

(329) **Kata, M., Giordano, F., Hadi, I. A., and Selmeczi, B.,** Conditions of cyclodextrin complexation, *Acta Pharm. Hung.*, 63, 285, 1993.

(330) **Brown, S. E., Coates, J. H., Easton, C. J., Lincoln, S. F., Luo, Y., and Stephens, A. K. W.,** Cyclodextrin inclusion complexes of two nonsteroidal antiinflammatory drugs and of an analgesic drug, *Aust. J. Chem.*, 44, 855, 1991.

(331) **Blanco, J., Vila-Jato, J. L., Otero, F., and Anguiano, S.,** Influence of method of preparation on inclusion complexes of naproxen with different cyclodextrins, *Drug Dev. Ind. Pharm.*, 17, 943, 1991.

(332) **Ahmed, M. O., Nakai, Y., Aboutaleb, A. E. S., Yamamoto, K., Abdel Rahman, A. A. Z., and Saleh, S. I.,** Complex formation of nitrazepam in coprecipitating and in co-grinding with methylated ß-cyclodextrins, *Chem. Pharm. Bull.*, 38, 3423, 1990.

(333) **Hirayama, F., Kurihara, M., and Uekama, K.,** Mechanisms of deceleration by methylated cyclodextrins in the dehydration of prostaglandin E2 and the isomerization of prostaglandin A2 in aqueous solution, *Chem. Pharm. Bull.*, 34, 5093, 1986.

(334) **Blecher, L., Ohmae, T., and de Jong, H. H. J.,** Tri-PEC: Reports from IPEC-Americas, JPEC, and IPEC Europe, *Pharm. Technol.*, 18, 53, 1994.

(335) **Mills, C. O., Martin, G. H., and Elias, E.,** The effect of tyrosine conjugation on the critical micellar concentration of free and glycine conjugated bile salts, *Biochim. Biophys. Acta*, 876, 677, 1986.

(336) **Badawy, S. I. F., Marshall, A. L., Ghorab, M. M., and Adeyeye, C. M.,** A study of the complexation between danazol and hydrophilic cyclodextrin derivatives, *Drug Dev. Indust. Pharm.*, 22, 959, 1996.

(337) Captisol™, Sulfobutylether ß-cyclodextrin: Product literature, CyDex, L. C., 7304 West 130th St., Suite 370, Overland Park, KS 66213-2627, (913) 685-8850, cydex@aol.com.